THE UNIVERSITY OF ALBERTA

THE INFLUENCE OF CARBON DIOXIDE AND SPECIFIC INORGANIC CATIONS ON VASCULAR SMOOTH MUSCLE CONTRACTILITY

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ABSTRACT

In intact dogs and cats the peripheral vascular responses to noradrenaline (NA) are depressed during hypercapnia. The purpose of this investigation was to determine if a similar depression could be induced by high pCO_2 in isolated vascular tissues and, if so, to study its mechanism.

It is postulated that the hypercapnic depression of the contractility of vascular smooth muscle is due to the effect of carbon dioxide or low pH on certain cations which are implicated in the contractile process. To test this hypothesis, experiments were performed to observe the log dose-response relationship of loops of rabbit aorta to noradrenaline when the tissues were exposed to solutions of different pH, pCO_2 and ionic concentrations.

The length of time of exposure of the smooth muscle to a high pCO_2 before the addition of noradrenaline to the bath was varied and it was found that as long as the exposure was kept fairly short a hypercapnic depression of the noradrenaline response was observed. In solutions of normal ionic composition the hypercapnic depression of contractility was overcome by high dose levels of noradrenaline but when the calcium ion concentration of the bathing medium was decreased the depression due to hypercapnia increased and was no longer overcome by raising the NA dose. When a low pH was induced in a bicarbonate-free medium in the absence of CO_2, a small depression of the contractility of the aorta was

INTRODUCTION

The purpose of this investigation was to determine the mechanism of the hypercapnic depression of the contractile response of arterial smooth muscle to noradrenaline and to investigate the significance of certain cations in the activity of the smooth muscle cell. Specifically, the object was to determine whether the depressant effect of excess carbon dioxide acted by an interference with the normal function of an essential cation.

Hypercapnia, in an intact animal, causes an increase in blood pressure and a decrease in the pressor response to noradrenaline (Shivak, 1961). In isolated preparations of vascular smooth muscle, hypercapnia has been shown to exert a depressant effect (Tobian, Martin, Eilers, 1959; Halpern *et al.*, 1959). However, it has not been clearly demonstrated whether the effect of carbon dioxide is due simply to the depressant effect of a decreased pH or whether the carbon dioxide effect is at least partially independent of the pH effect.

It has been shown that respiratory acidosis is accompanied by an increase in an animal's level of circulating catecholamines (Miller, 1960). Possibly, it is this increase which is responsible for the temporary increase in blood pressure which hypercapnia induces. It was postulated by Burn that the effect of an increased catecholamine level is only temporary because of a saturation of adrenergic receptors, leaving few receptors free on which catecholamines present in the blood stream may act

(Burn, Rand, 1959). Shivak tested the hypothesis that the increased catecholamine level of the blood in hypercapnia might be responsible for such a "saturation of adrenergic receptors" resulting in the hypercapnic depression. The hypothesis was rejected after it had been tested in two series of perfusion experiments in reserpinized and unreserpinized dogs and cats. Shivak concluded that the depression was effected at some point in the contractile process beyond the postulated adrenergic receptor site (Shivak, 1961).

Calcium ions appear to be essential for smooth muscle contractility whatever the method of stimulation might be (Bohr, Goulet, 1961; Briggs, 1961; Daniel, Sehdev, Robinson, 1962). The degree of tonic shortening and the excitability of the arterial smooth muscle cell depends upon the K_i/K_o ratio (Barr et al., 1963). Smooth muscle activity may also be affected by the other major inorganic cations sodium and magnesium, present in a physiological solution. In the case of vascular smooth muscle, there is evidence which suggests that both these ions compete with calcium at the cell membrane (Bohr, Goulet, 1961; Briggs, Melvin, 1961) although a sodium-calcium antagonism was not demonstrated in the uterus (Daniel et al., 1963).

The present investigation was carried out on isolated loops of rabbit aorta, so that the effects of pCO_2 and pH changes, as well as ion concentration changes, could be observed directly on the smooth muscle. For this investigation, in order to obtain data more efficiently, new apparatus was developed so that tension recordings could be obtained from eight tissues simultaneously.

LITERATURE REVIEW

Properties of Arterial Muscle

For over fifty years, rings, strips, or segments of larger blood vessels have been used for qualitative and sometimes for quantitative studies of the effects of various drugs on vascular smooth muscle in vitro. The preparation most commonly used is the spiral strip of rabbit thoracic aorta, which is sensitive to many drugs and gives reproducible responses to small test doses over a period of many hours.

Furchgott has explained, in considerable detail, the preparation of the aorta and the apparatus required to measure tension changes. He has observed that the time required for contraction and relaxation of the tissues in the bath depends upon the concentration of the stimulating agent added. The time required to produce a maximum contraction varies from one to fifteen minutes and the time required to produce complete relaxation is ten to sixty minutes. Reproducible results are obtainable as long as contractions are less than half-maximal. The sensitivities of strips vary considerably, but the range of noradrenaline concentrations to which the tissues respond is nearly constant from 3×10^{-10}, for the first detectable contraction, to 10^{-5} for a maximal contraction (Furchgott, 1960).

In most arteries, including the rabbit aorta, the circularly oriented smooth muscle constitutes almost half the contents of the vessel wall (Furchgott, 1960). Therefore, the direction of cutting of the smooth muscle is important. Most of the work done on isolated rabbit aorta has

been done on spirally cut strips (Furchgott, 1960; Sparks, Bohr, 1962; Tobian et al., 1959).

Contractility of vascular smooth muscle increases with stretch over a certain range. Using dog superior mesenteric artery, Sparks and Bohr studied the effect of stretch on the magnitude of tension developed in response to a standard submaximal stimulus. They found that with each increase in length, there is a corresponding increase in the magnitude of the response, until an optimal length is reached. With further increases in length, there is a progressive decrease in response. Responses to such dissimilar stimuli as adrenaline and electric current are similarly influenced, suggesting that the mechanism of the influence is a basic component of the contractile machine shared by the responses to all agents (Sparks, Bohr, 1962).

Excised rat aorta placed in a bath loses over two-thirds of its potassium and gains roughly an equivalent amount of sodium. This seems to be due to mechanical handling of the tissue so it is essential that the tissues be allowed to remain in the organ bath for one or two hours, so that they can recover the normal sodium-potassium gradient across the membrane (Dawkins, Bohr, 1960).

Buffer Systems

A buffer is defined as any substance in a fluid which tends to lessen the change in the hydrogen ion activity which would otherwise be produced by the addition of acid or alkali to the fluid. Some of the more effective physiological buffering systems are the haemoglobin, bicarbonate and phosphate buffers of the blood plasma (Davenport, 1958).

Carbon dioxide (CO_2) is one of the gases with which the body must deal. It is constantly being produced as a result of metabolism and when an excess amount of it is present, the condition of "respiratory acidosis" develops. Dissolved CO_2 reacts with water according to the equation $CO_2 + H_2O \rightleftharpoons H_2CO_3$ (1). The equilibrium is far to the left, but the small amount of H_2CO_3 formed ionizes according to the equation $H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$ (2).

For weak electrolytes, the relation between the substances represented in the equation can be expressed by the mass action law:

$$K = \frac{[H^+]\ [HCO_3^-]}{[H_2CO_3]} \quad (3).$$

If one assumes that the equilibration of CO_2 with water is instantaneous and that the activity of water is sufficiently in excess of that of all other constituents, so that it can be considered to be a constant, then one can assume that the concentration of carbonic acid is proportional to the concentration of dissolved CO_2. Equation (3) will then give

$$K^1 = \frac{[H^+]\ [HCO_3^-]}{[CO_2]} \quad (4).$$

Since $[H^+]$ is expressed in terms of pH, this equation is modified by taking logarithms.

$$-\log [H^+] = -\log K^1 + \log \frac{[HCO_3^-]}{[CO_2]} \qquad (5).$$

Since $-\log [H^+]$ is the definition of pH, the -log K is the definition of pK, so that $pH = pK + \log \frac{[HCO_3^-]}{[CO_2]}$ (6).

Normal blood bicarbonate is 27 meq. per litre and the carbonic acid concentration is 1.35 meq. per litre. Therefore, the normal HCO_3^- / CO_2 ratio is 20/1. If the ratio is preserved at this value, the pH will be normal, no matter what changes in concentration occur. A decrease in the ratio causes a decrease in pH or acidosis. This type of acidosis can occur when there is an excess of CO_2 in the body, or in the bathing fluid when one is dealing with preparations of isolated tissue (Davenport, 1958).

Effect of CO_2 and pH on Smooth Muscle

Blood vessel calibre, as well as most smooth muscle activity, is affected by variation of the concentration of hydrogen ion in the tissue bathing medium.

As early as 1920, it was reported that the injection of sufficient sodium carbonate to make dogs alkalotic was followed by increased responses to adrenaline, while the intravenous injections of acid sodium phosphate antagonized the pressor effect of adrenaline (Collip, 1921).

In the pithed cat, the blood pH could be varied between 6.9 and 8.0 by varying the amount of air carried to and from the lungs. Small intravenous injections of adrenaline were found to act with progressively increasing effectiveness as the pH increased (Burget, Visscher, 1927).

It was reported in 1957 that, when sympathomimetic amines were administered to dogs under conditions of respiratory acidosis, the pressor responses were somewhat diminished, as compared to those during the control state at a normal pH. When the agents were administered to dogs subjected to a total cardiac by-pass, pressor responses under conditions of respiratory acidosis were uniformly much less in these animals than the control responses (Houle *et al.*, 1957).

Fleismann *et al.* reported that segments of large and small arteries constitute independent resistances, whose magnitude may actively vary in opposite directions. Using a multiple stopcock arrangement, these workers measured the pressures rapidly and consecutively in the dog forelimb vascular system in the brachial artery, a small artery, a small vein and in the cephalic vein. They found that an acute increase

in $[H^+]$ is associated with small vessel dilatation and simultaneous large artery constriction. A decrease in $[H^+]$ is associated with small vessel constriction and large arterial dilation. The overall result is little change in the total resistance (Fleismann et al., 1957).

Duner and von Euler observed an increase in cat hind limb blood flow and blood pressure during the infusion of noradrenaline into the femoral vein, but these flow and pressure changes were reduced during hypercapnia (Duner, von Euler, 1959). In order to exclude all possible routes of collateral circulation, von Euler used isolated limb preparations in which the bone marrow was plugged and ligatures were placed on the upper thigh, so that only the femoral and sciatic nerves and femoral vessels were functional. With such a preparation, even moderate degrees of respiratory acidosis, induced by the breathing of CO_2, diminished the pressor effect of noradrenaline (Bydgeman, von Euler, 1962).

Similarly, Nash and Heath observed that there was a reduced peripheral vascular response to adrenaline and noradrenaline in dogs subjected to hypercapnia and, consequently, a low blood pH. During hyperventilation and a high blood pH, the vascular response to these drugs increased (Nash, Heath, 1961).

By using dichloroisoproterenol (DCI) and phentolamine, Wood et al. were able to eliminate the depressor and pressor components of the adrenaline response selectively. They observed, in dogs, that hypoxia and respiratory acidosis can reduce the amplitude of both the pressor and depressor responses to adrenaline (Wood et al., 1963).

Shivak postulated that an intrinsic catecholamine release by hypercapnia causes a partial saturation of adrenergic receptors and is

responsible for the lowered sensitivity to noradrenaline which is observed during hypercapnia. If this postulate is correct, reserpine should abolish the hypercapnic depression, because of its effect on noradrenaline stores in tissue. Since this effect was not observed in either dogs or cats, he concluded that the depression occurred beyond the receptor level, possibly on membrane permeability (Shivak, 1961).

A number of workers have observed the effect of pH changes on isolated strips of rabbit aorta suspended in Kreb's solution, which simulated conditions of respiratory and metabolic acidosis and alkalosis. They reported that the contractions of the strips in response to noradrenaline were maximal in states of relative alkalosis and minimal in states of relative acidosis. In "respiratory acidosis", inhibition averaged 43%; in "metabolic acidosis" it averaged 26% (Tobian, Martin, Eilers, 1959). Williamson and Moore varied pH by varying the relative proportions of sodium chloride and sodium bicarbonate. They found that the tissue response to noradrenaline was decreased approximately 25%, both when the pH was reduced from 7.43 to 6.80 and when it was raised from 7.43 to 7.70 (Williamson, Moore, 1960).

Halpern and Binaghi separated a high CO_2 effect from a low pH effect when they observed the effect of H_2CO_3 on the stimulating action of histamine on the guinea pig intestine and oxytocin on the uterus. The H_2CO_3 inhibited the action of histamine and oxytocin, but had little or no effect on the activities of acetylcholine, potassium chloride and the ergot alkaloids under the same conditions. The inhibitory action of the H_2CO_3 is directly proportional to its concentration in the medium. The activity of the histamine and oxytocin is not affected at different

pH values in a bicarbonate free medium which is buffered with a mixture of sodium maleate and maleic acid. This suggests that the inhibitory effect of the H_2CO_3 is specific and independent of simultaneous modifications in the hydrogen ion concentration (Halpern et al., 1959).

Ions and Smooth Muscle

According to the classical ionic hypothesis, an action potential consists of an initial large, brief inward sodium current, followed by an outward potassium current. The action potential slightly lowers the large ionic gradient present across the cell membrane. If the cell is metabolizing, the sodium-potassium pump restores the gradients over a period of time. A number of other ions commonly bathe the body cells, but their significance in the environment of the cells remains, for the most part, unknown.

Calcium ions have been implicated in the contractions of muscles in response to stimulation. The concentration of calcium required to produce a given response in atrial muscle is reduced two-to three-fold in the presence of adrenaline (Briggs, Holland, 1960). Calcium ions appear to be essential for smooth muscle contraction, whatever the method of stimulation may be, (Bohr, Goulet, 1961; Briggs, 1961; Daniel _et al._, 1962).

The initiation and the maintenance of contraction are associated with an increased calcium influx in normal rabbit aorta (Bohr, Goulet, 1961; Briggs, 1961) and in cat ileum (Evans _et al._, 1958). Efflux, however, is reported to be unaffected by these stimuli (Briggs, Melvin, 1961). Briggs found that, during a K_2SO_4 induced contraction in rabbit aorta, there is a linear relationship between the tension developed and the rate of entry of Ca^{45}. However, he was unable to detect a change in the concentration of tissue calcium following such a contraction. He suggested the possibility of an increased calcium exchange (Briggs, 1961).

This suggestion does not follow his observation that there was no increase in the Ca^{45} efflux during stimulation.

Using the taenia coli of the guinea pig, Schatzmann could detect no increase in the level of tissue calcium when the muscle was stimulated with acetylcholine or potassium chloride. The efflux of Ca^{45}, however, did increase. He assumed that the EDTA titration method was not sensitive enough to measure tissue calcium accurately (Schatzmann, 1961).

Hinke and Wilson agreed that calcium is essential for muscle contractility, but they did not assume that all stimulating agents made use of calcium in the same way. In an isolated arterial segment, the contraction is response to noradrenaline and pitressin appears as a maximal contraction between 0.5 and 0.75 mm/litre of calcium. With a potassium contraction, the response continues to increase with the concentration of calcium. With a potassium contracture, the response continues to increase with the concentration of calcium. They interpreted this to mean that calcium which enters during potassium depolarization probably comes from free calcium in the extracellular space because the resulting contracture is proportional to the calcium concentration. Calcium which enters on drug excitation, on the other hand, may be released from bound membrane calcium (Hinke, Wilson, 1963).

Daniel _et al._ presented evidence that calcium is the essential link between excitation and contraction in both polarized and depolarized rat uterine muscle. It was suggested that, under normal conditions, in smooth muscle, drugs may initiate contraction by the release of calcium from binding sites in the membrane or elsewhere and by increase in the permeation of calcium through the membrane. Strontium and barium are

able to substitute for calcium, possibly because of increased permeation, although they are not tightly bound (Daniel, Sehdev, Robinson, 1962).

Using guinea pig taenia coli, Schatzmann reported the existence of three compartments from which Ca^{45} emerged in washout. The $t_{1/2}$ values were found to be less than three minutes, three minutes and thirty minutes, (Schatzmann, 1961). Using strips of cat ileum, Sperelakis found the washout curves to be composed of two exponentials with $t_{1/2}$ values of eight minutes and sixty minutes (Sperelakis, 1962). The first fraction reported by Schatzmann and probably ignored by Sperelakis is attributed to the extracellular water space and the other two are attributed to the cell surfaces and the intracellular space, respectively.

Bohr observed that a vascular smooth muscle response to adrenaline is differentiable into a fast (F) and a slow (S) component. The F component is completed within forty-five to sixty seconds after the initial stimulation, while the S component persists throughout the remainder of the contraction period. The F component is the larger in conditions of low external calcium concentration $[Ca^{++}]_o$ and is depressed as $[Ca^{++}]_o$ increases. The S component, on the other hand, is usually absent when $[Ca^{++}]_o$ is less than 0.3 mM and reaches its maximum when it is 1 mM. He postulated that membrane excitability governs the F component so that, as the external calcium concentration increases, the membrane is stabilized. On the other hand, the rate limiting factor for the S component could be the availability of calcium for the coupling process (Brodie *et al.*, 1957; Brodie *et al.*, 1959).

When stored in the cold, artery strips lose their ability to respond to a stimulus. This can be correlated with the loss of potassium which occurs in a cold environment in isolated tissues (Barr, Headings, Bohr, 1963). The $[K^+]$ bathing the smooth muscle tissue appears to be involved in determining the muscle's capacity to respond to a stimulus. The adrenaline response in isolated rabbit aorta strips varies directly with the $[K^+]$ in the bathing medium (Bohr, Brodie, 1958). In smaller resistance vessels, however, the potassium effect is biphasic, small increases depressing and large increases potentiating the effect (Frohlich et al., 1962).

Catecholamines and possibly other excitatory stimuli have an effect upon the intracellular $[K^+]$ of smooth muscle cells. Daniel et al. reported that, in rat aorta, noradrenaline produces a depletion of aorta potassium, while sodium and water move in secondarily (Daniel et al., 1957). Barr observed that K^{42} efflux from arterial smooth muscle increased when the muscle was stimulated with adrenaline, histamine, potassium, or electrical stimulation (Barr, 1961). Headings and Rondell, however, were able to demonstrate a net K-efflux from vascular smooth muscle accompanying tension development by electrical, but not by catecholamine, stimulation. Instead, they reported a net K-influx accompanying catecholamine stimulation (Headings, Rondell, 1962). Bohr could not observe a net entry of Na^{24} into rabbit aorta during catecholamine stimulation (Bohr, Goulet, 1961).

Strips of dog carotid artery which have been stored at 4° C for a long time contract when placed in recovery solutions at 38° C. This initial contraction is not related to K_o, but the subsequent relaxation

is greater in rate and magnitude as K_0 is increased. When K_0 is zero, relaxation does not occur. Although K_i is higher as K_0 is higher, the K_i/K_0 ratio is lower. Evidence points to the fact that the high K_0 contracture depends on the K_i/K_0 ratio. This contracture is greater when the ratio is lower, which suggests that it is a manifestation of increased membrane permeability. Barr et al. concluded that (1) increased K_i increases contractility; (2) increased K_i increases the relaxation rate; (3) excitability is decreased by too high or too low a K_i/K_0 ratio; and (4) the extent of tonic shortening depends on the K_i/K_0 ratio (Barr et al., 1963).

It has been reported that an increase in $[Na^+]_0$ is responsible for a decrease in the responsiveness of smooth muscle (Williamson, Moore, 1960; Briggs, Melvin, 1961), and that a decrease in $[Na^+]_0$ causes an increase in smooth muscle responsiveness in both resistance and conduit vessels (Bohr, Brodie, 1958; Hughes et al., 1956). Hinke and Wilson stated that, in the presence of low Na_0, contraction was potentiated during potassium depolarization, but not when noradrenaline was the stimulating agent (Hinke, Wilson, 1963).

It has been suggested that the changes in contractility induced by varying $[Na^+]_0$ appear to act through a sodium-calcium antagonism (Bohr, Goulet, 1961). Briggs reported that, when the sodium chloride concentration in the solution bathing rabbit aortic strips was reduced, Ca^{45} influx in the aorta increased 225% when it was stimulated by adrenaline. In a normal bathing medium, the influx increased only 105% (Briggs, Melvin, 1961). However, using rat uterine muscle, Daniel could not demon-

strate such an antagonistic effect. He found that the shortening on elevation of the potassium concentration is often enhanced by increasing the sodium concentration while that in response to acetyl choline is reduced. In uterine muscle, sodium prevents relaxation as long as the potassium concentration remains elevated. An increase in the sodium concentration can produce a contraction of depolarized smooth muscle.

Neither sodium nor chloride ions appear to be essential for an adrenergic response. In intestinal arteries, Waugh demonstrated that a contractile response could be obtained even when sodium chloride had been completely replaced by lithium chloride or sucrose (Waugh, 1962).

It appears that the adrenergic neurohormone excites both depolarized and previously polarized vascular smooth muscle by the same membrane mechanism of calcium release, the calcium moving intracellularly to activate contraction (Waugh, 1962).

Drug Antagonism

If the effect of a drug (size of contraction) is plotted against the log of the dose, the curve obtained is essentially sigmoid shaped. Two main theories have been proposed to account for this shape: 1. The first theory, proposed by Clark, is that the relation between dose and effect depends upon an equilibrium between the rate at which the drugs combine with receptors and the rate at which they disappear from receptors. 2. The second is that the response of the whole tissue is the sum of the responses of a large number of small elements responding independently to the drug and the shape of the curve depends upon the distribution of the sensitivities of those elements.

Clark, in order to work out an equation relating the concentration of an applied drug and its action on a tissue, made the simplifying assumptions that the reaction between an active drug and its specific receptors is reversible and obeys the law of mass action; that the receptors are all uniform in their affinity for the drug; and that the magnitude of the response elicited by the drug is proportional to the fraction of the total number of receptors combined with the drug. He also assumed that, when different active drugs (agonists) with different affinities for the same specific receptors, combined with the same fraction of receptors, equal responses would be obtained; when antagonists combined with receptors in the absence of agonists, no response would be detectable. This assumption demands an all or none action on receptors by drugs combined with them and an equal response to the combination of agonist with any receptor.

The Michaelis-Menten theory of enzyme action is expressed in the

relationship $E+S \rightleftharpoons ES \rightleftharpoons E+P$ where E is the free enzyme, S the substrate and P the products of the reaction. The kinetics are characterized by the equation $V = \frac{V_{max}\,[S]}{K_m + [S]}$ where V is the measured velocity of the reaction at substrate concentration $[S]$, V_{max} is the limiting velocity and K_m the Michaelis constant.

This equation will be recognized as being identical in form to the drug receptor equation of Clark, which is $A = \frac{A_m\,[D]}{K_D + [D]}$ where A is the measured action, A_m is the maximal response possible (all receptors occupied), D is the concentration of free drug, K_D is the dissociation constant of the drug-receptor complex.

If A is plotted against log D, the familiar sigmoid curve mentioned above is obtained. If 1/A is plotted against 1/D, a straight line is obtained with a slope equal to K_D/A_M and an intercept equal to $1/A_m$ (Gaddum, 1957).

Mounter and Turner point out that, with the Michaelis-Menten equation, a transformation introduced by Eadie is to be preferred to the commonly used Lineweaver and Burke transformation. In Eadie's transformation, V/S is plotted against V. The slope is given by $-K_m$ and the intercept is V_m. The advantages of this form are (a) it has finite intercepts on each axis; and (b) the points are more evenly distributed than those in the Lineweaver and Burke transformation.

If applied to Clark's equation, which is discussed above, A/D is plotted against A. The slope is given by $-K_D$ and the intercept is A_m [(Mounter, Turner, 1963)].

Gaddum states that drug antagonism can doubtless be due to a

number of independent mechanisms:

1. Independent antagonism which occurs when drugs have independent, opposite effects.
2. Antagonism by neutralization, in which the two drugs combine with each other to form an inactive compound.
3. Non-competitive antagonism, in which all responses are reduced by a constant proportion.
4. Competitive reversible antagonism, in which drugs compete directly for the receptor site.

The theory of competition to which Clark's equation applies, is one very commonly used. Recent work, however, has revealed facts which cannot be explained by this theory in its simplest form. Gaddum outlines these facts as follows:

(a) The shape of the log-dose effect curve is often different from that predicted by theory.

(b) The maximum effect in the presence of the antagonist may be less than the maximum effect in its absence.

(c) The presence of the antagonist may alter the slope.

(d) The effects of some antagonists (e.g. ergotamine on adrenaline receptors on rabbit uterus) develop slowly and disappear slowly when the drug is removed from the bath.

(e) Some drugs may produce a small effect and yet block the action of other drugs (Gaddum, 1957).

Furchgott has tested the applicability of Clark's equation in the case of strips of rabbit aorta. Experimental curves (plotted as isotonic contraction height against log concentration) obtained in a first adrenaline

"concentration series" on individual strips, usually deviated considerably at higher levels of contraction from a theoretical curve, but experimental curves obtained in a second "concentration series" on the same strips often closely approximated a theoretical curve. Noradrenaline gives a concentration action curve quite similar to that for adrenaline.

Furchgott states that, in view of all the simplifying assumptions made by Clark, it is surprising that so much of the data obtained in the concentration-action experiments on smooth muscle fit even reasonably well the theoretical curves of the equation. Even if the same dissociation constant applies to the reaction of all the specific receptors with a specific drug, and if maximal response occurs when all the receptors are combined with the drug, it still seems impossible that the response should be proportional to the fraction of total receptors combined with the drug. Rather, a lack of proportionality would be expected because the drug-receptor reaction is only the first step in a complex process leading to a response. In the case of smooth muscle contraction, it is likely that the primary drug-receptor reactions lead to a chain of reactions which terminate in the activation of the contractile process (Furchgott, 1955).

A more recent theory on the mechanism of drug action has been advanced by Paton, who states that many of the phenomena of drug action can be interpreted on the assumption that the stimulant effect of a drug depends, not on the number of receptors occupied, but rather on the rate of occupation. He postulates that chemical stimulation readily produces, in addition to changes in receptor state, a considerable non-specific change in tissue responsiveness.

Both association and dissociation occur according to mass action

law and stimulant action is proportional to the rate of association between drug molecule and receptor. Agonist drugs dissociate rapidly from receptors while antagonist drugs dissociate slowly (Paton, 1961).

METHODS

Series I. Experiments with Whole Animals

Cats were anaesthetized with 50 mgm/kgm nembutal, injected intraperitoneally. A tracheal cannula was inserted and the left jugular vein was cannulated for the administration of heparin and anesthetic as they were required. The carotid artery was cannulated and the blood was diverted through a Sigmamotor pump and returned to the abdominal aorta above the renal artery.

To prevent blood clotting, a 4 mgm/kgm initial dose of heparin was given intravenously and this was supplemented by a 1 mgm/kgm intravenous dose every thirty minutes. The responsiveness of the vascular bed was determined by measuring the blood pressure changes induced by intra-arterial doses of noradrenaline at dose levels of 62.5, 125, 250, 500 and 1,000 ng/ml. Noradrenaline dilutions were made in a saline solution containing 0.05% sodium metabisulfite to act as a preservative. The drug was injected in a 0.1 ml volume through a rubber stoppered side-arm of the Sigmamotor Pump Circuit. Two T-tubes were present in the circuit, one above and one below the pump to connect pressure transducers which recorded the systemic pressure of the animal and the perfusing pressure to the hind limbs, respectively. The pressures were recorded on a Grass Polygraph.

The doses of noradrenaline were administered to the cats while they were breathing a 95%O_2-5%CO_2 gas mixture and again after they had

been made hypercapnic by breathing a $70\% O_2$-$30\% CO_2$ gas mixture.

Spinal Cats

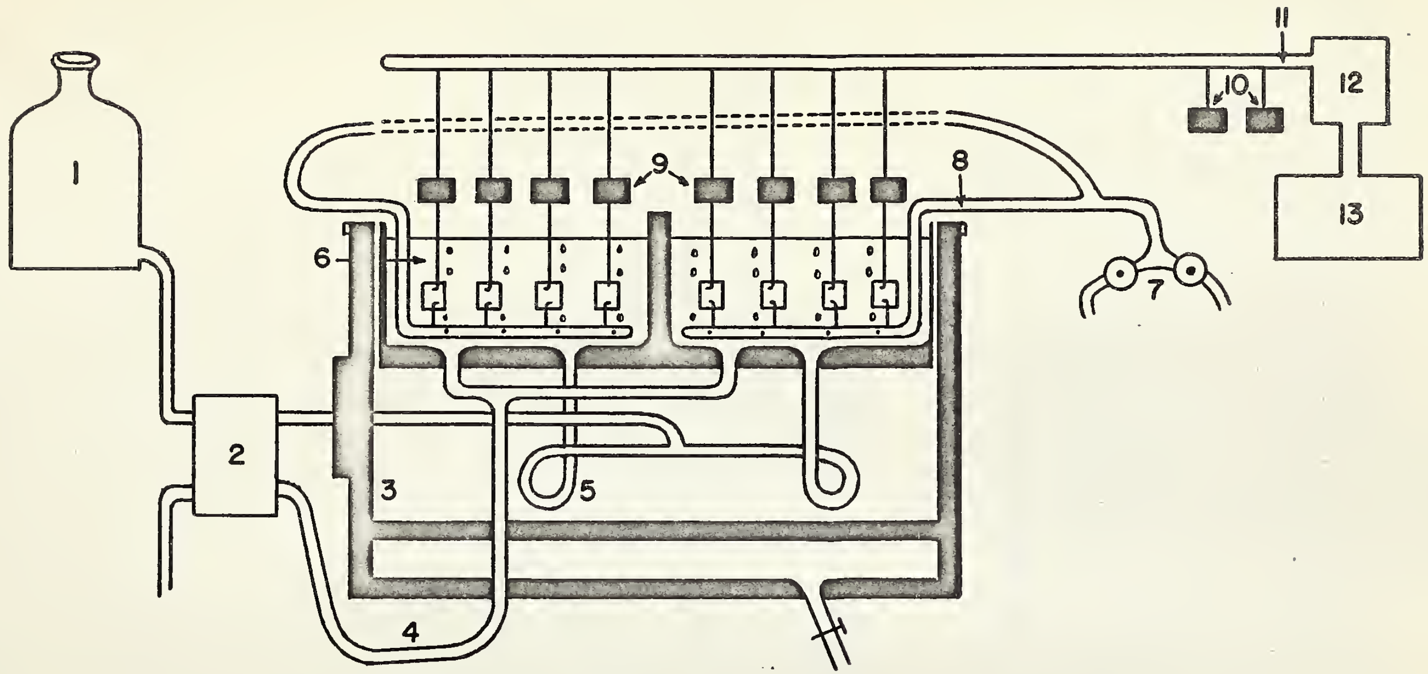
Another group of cats were anaesthetized as described above and the tracheal and jugular cannulae were inserted. The animals were artificially respired. The carotid and mesenteric arteries were isolated, but not cannulated. The animals were spinalized, using the method described by Burn (Burn, 1952). The remaining surgery was completed and the noradrenaline responses were measured in normal and hypercapnic animals, as described above.

Ganglion Blocked Dogs

Dogs were anaesthetized with 35 mgm/kgm of nembutal, injected intravenously. The animals were prepared in the same manner as the first group of cats described above. Pentolinium tartrate (ansolysen) was injected intravenously in a 1 mgm/kgm dose to produce a ganglion block. The blood was diverted from the carotid artery and pumped back into the carotid artery, in order to perfuse the head.

Figure 1·

ORGAN BATH and RECORDING SYSTEM



1· Reservoir for Kreb's Solution
2· Automatic Control Valve
3· Temperature Controlled Palmer Water Bath
4· Drain
5· Heating Coils
6· Organ Bath (Perspex)
7· Manifold for Gas Tanks
8· Aeration Tube and Anchor for Tissues
9· Strain Gauges on Ratchets
10· Strain Gauges - Baseline Control
11· Cable
12· Stepper Relay and Balance Bridges
13· Single Channel Texas Recorder (10" paper)

Series II. Isolated Loops of Rabbit Aorta

Two plastic muscle chambers having dimensions of about 1 x 4 x 6 cms. and capacities of twenty-five mls. were suspended in a water bath maintained at 37° C. Inlet openings at the bottom of these chambers were connected through rubber tubing and glass heating coils to an overhead reservoir containing the solutions which were used to bathe the tissues (Appendix I). The bath was filled and emptied automatically by an electrically-controlled double-valved apparatus, which operated on a three-minute cycle.

In order to aerate the bath, glass tubing, having very fine perforations, was bent to fit the bottom of each bath and attached through rubber tubing and pressure control valves to tanks containing the required gas mixtures. To attach the tissues at the bottom of the bath, four stainless steel hooks were imbedded in each aeration tube so that eight loops of aorta tissues could be suspended in the two baths. The upper ends of the aorta loops were also suspended on stainless steel hooks attached to strain gauge force displacement transducers. Each strain gauge was supported on a rack and pinion to allow adjustment of the tension applied to the tissue. This apparatus is illustrated in Figure 1.

The strain gauges were connected through an eleven place stepper relay system to a wide tape, single channel Texas Recorder, so that a measure of the tension on each tissue loop was recorded in succession with each cycle of the relay. The duration at each contact could be varied from three to five seconds. The three-second duration was found

to be satisfactory for clear records. Thus, the cycle was repeated every thirty-three seconds. Because the aorta is a relatively slowly responding tissue, this frequency was found to be satisfactory to record the peak response of each of the tissues. The recorder was calibrated so that a one gram change in tension produced a one inch deflection on the recording paper. The record was analyzed by tabulating the peak deflection for each tissue at each dose level and calculating the mean response of all the similar tissues at each dose level.

Noradrenaline bitartrate was added to each bath in a 0.5 ml. volume from a tuberculin syringe. The final concentration of the drug in the organ bath was varied in ten steps from 10^{-9} to 10^{-5} grams/ml. The noradrenaline dilutions were made in isotonic saline solution containing 0.05% sodium metabisulfite as a preservative. The noradrenaline was added to the bath as soon as the bath had filled with fresh bathing solution and it was left in contact with the tissues until the bath was emptied during the automatic cycle, that is, for three minutes.

Preparation of the Aorta Loops

Rabbits were killed by a blow on the back of the head. The thoracic aorta was removed as quickly as possible, care being taken to prevent stretching it. Fat and connective tissue were trimmed off carefully. Loops of 2 to 3 mm. in width were cut and suspended in the bathing solution in the bath. Each tissue was attached to its respective strain gauge and the basal tension on the loop was adjusted to four grams. The tissues tended to relax during the 1½ hour equilibration period, so the tension was adjusted at intervals during this period.

Modifications of the Bathing Solution

Group I. pH Variation with CO_2

The organ bath was filled with Kreb's bicarbonate solution (Appendix II). When the gas mixture was 95%O_2-5%CO_2, the pH of the Kreb's solution in the bath varied between 7.3 and 7.5, but when the gas was 70%O_2-30%CO_2, the pH of the solution varied between 6.7 and 6.9. The contraction produced by each dose of noradrenaline was observed during aeration with both the gas mixtures before a higher dose was added. After a dose of the drug, the tension of the tissues was allowed to return to its four gram tension base line before it was again stimulated.

Modifications of the Kreb's bicarbonate solution were made in a number of ways, but the same experimental procedure for changing pH and dose order was used in each experiment. Modifications made included the following:

Variation of Calcium	- 1/16, 1/8, 1/4, 1/2, 2 x normal Ca.
Variation of Potassium	- 1/16, 1/8, 1/4, 1/2, 2 x, 4 x normal K
Variation of Magnesium	- Mg-free and 2 x, 4 x normal Mg.
Variation of Sodium	- 1/2 Na - 1/2 Sucrose, 1/2 Na - 1/2 li, 1/4 Na - 3/4 Sucrose.

Group II. pH Variation Without Carbon Dioxide or Bicarbonate

The organ bath was filled with Kreb's solution from which sodium bicarbonate had been eliminated and in which the sodium chloride concentration was raised, in order to bring the sodium concentration back to its normal level. The pH was adjusted to 7.4 (Solution 1) and 6.75 (Solution 2) with normal sodium hydroxide. The contraction produced by each

addition of noradrenaline was again observed at each pH level before a higher dose level of the drug was added. The ion concentrations were modified in the same way as those of Group I.

After each addition of noradrenaline in the experiments of Groups I and II, the pH of a sample of the bath solution was measured at 37^{0} C. with a Metrohm pH metre.

Two slightly different procedures for the addition of noradrenaline to the organ bath were used:

Dose	Procedure I	Procedure II
10^{-9} gm/ml	High pH solution or 5% CO_2	High pH solution or 5% CO_2
10^{-9} gm/ml	Low pH solution or 30% CO_2	Low pH solution or 30% CO_2
5 10^{-9} gm/ml	Low pH solution or 30% CO_2	High pH solution or 5% CO_2
5 10^{-9} gm/ml	High pH solution or 5% CO_2	Low pH solution or 30% CO_2
10^{-8} gm/ml	High pH solution or 5% CO_2	High pH solution or 5% CO_2
10^{-8} gm/ml	Low pH solution or 30% CO_2	Low pH solution or 30% CO_2
5×10^{-8} gm/ml	Low pH solution or 30% CO_2	High pH solution or 5% CO_2

With Procedure I, the tissues were in contact with the 30% CO_2 or low pH solutions for longer periods of time (15-60 minutes) before they were stimulated, while with Procedure II the exposure of the tissues to the low pH solutions was for a shorter duration (3-15 minutes).

Group III. Ionic Alterations and Contractility

The tissues were bathed in Kreb's bicarbonate solution and respired with a $95\%O_2$-$5\%CO_2$ gas mixture. Contractions in response to noradrenaline were compared in the following solutions:

Normal solution and Mg-free solution

Normal solution and 4 x normal Mg solution

Normal solution and 1/2 Na - 1/2 choline solution

Normal solution and 1/4 Na - 3/4 choline solution

1 Na - 1/8 Ca solution and 1/2 Na - 1/8 Ca solution

1 Na - 1/8 Ca solution and 1/4 Na - 1/8 Ca solution

Normal solution and 1 Na - 1 Sucrose solution#

Normal solution and 2 Na solution#

Normal solution and 1 Na - 0.5 Sucrose solution#

Normal solution and 1.5 Na solution#

- hypertonic solutions.

Using one set of tissues, the responses to a particular dose level of noradrenaline were compared with different concentrations of calcium in the Kreb's bicarbonate bathing solution. A similar experiment was done with different concentrations of potassium in the bathing solution.

Because vascular smooth muscle is not rapidly depleted of its calcium, the loops of aorta were allowed to remain in contact with the solutions in which the calcium ion concentration was altered throughout the entire experiment. When the contractions were to be compared in solutions of different calcium ion concentration, the experiment was begun in a calcium-free solution and the concentration was progressively increased. The solution in the organ bath was changed at least every ten minutes.

When the magnesium, potassium and sodium concentrations were varied, the solutions were allowed to be in contact with the tissue loops

for about ten minutes before a dose of noradrenaline was added. The altered solutions were replaced by normal Kreb's solution as soon as the contraction had reached a maximum and the tissue was beginning to relax. This was done in order to prevent a drastic alteration of the intracellular ion concentrations.

At the conclusion of each experiment, each blood pressure response or the responses of each one of the eight isolated aorta loops was measured and the average response to each noradrenaline dose was calculated. Often the means were calculated using the data from two or more experiments which were done using the same experimental procedure. Each of the mean values obtained was plotted on semi-log graph paper to obtain a log-dose response curve and a best-fit curve was drawn through the points. If there was a separation of the points such that two best-fit curves could be drawn when contractility was being compared in two solutions or with two gas mixtures, it was assumed that contractility was affected by the presence of the altered ionic concentration or the CO_2 concentration.

The Standard Errors of the means of the two sets of points at each dose were calculated using the following formula:

$$SE = \sqrt{\frac{x^2 - \frac{(\Sigma x)^2}{n}}{n(n-1)}}$$

The Students' Paired t-test was used to calculate the significance of the difference between the two points which were being compared:

$$t = \frac{\frac{\Sigma(x_1 - x_2)}{n}}{\sqrt{\frac{\Sigma(x_1 - x_2)^2 - \frac{(\Sigma x_1 - \Sigma x_2)^2}{n}}{n(n - 1)}}}$$

P values were obtained from a corresponding table

The linear transformation of the dose response relationship is described on page 18 of the literature review. It was applied to the data when it was desirable to obtain the maximal response which the blood pressure response as isolated vascular tension tended to attain. When using the linear transformation proposed by Eadie, the y-axis intercept represents the maximal response (corresponding to A_m or V_m as outlined on page 18).

It has not been possible to find a satisfactory way to apply statistical tests to the transformed data.

RESULTS

Section I. Variation of CO_2 and pH

Whole Animal Experiments

A number of experiments were performed on intact animals in order to demonstrate the existence of a hypercapnic depression of the noradrenaline response and the fact that the depression is overcome by high dose levels of noradrenaline. The summary of the data is presented in Appendix II, Table 1. The linear transformations of the dose-response curves are shown in Figure 2, in which the response/dose ratio is plotted against the response.

The statistics in Table 1A support the conclusion of a decrease in response due to the hypercapnia. In Tables 1B and 1C the calculated statistical values do not indicate significant differences between individual sets of points. However, though this data does not give support to the conclusion that individual doses were significantly affected, there is nevertheless a depression at every dose. The linear transformations of the data as seen in Figures 2A and 2B suggest the existence of a trend to overcome the depression at high dose levels of noradrenaline since the graphs indicate that the maximum level of the blood pressure response is approximately the same at both the 5% and 30% levels of CO_2 in the respired gas. This trend is not indicated in Figure 2C.

Isolated Aorta Loops

The first response of the aorta loops to noradrenaline was usually

observed at the 5×10^{-9} gm/ml dose level and the maximum response occurred at the 5×10^{-6} gm/ml dose level. There was a great deal of variation in the responsiveness of the loops.

When Procedure I (see Methods) was used and noradrenaline added to the organ bath, a hypercapnic depression was not observed either in the normal Kreb's bicarbonate solution or in a solution in which the ion concentrations were varied. Figure 3 shows representative graphs. The corresponding data are in Appendix II, Tables 2 to 6. Subsequently, Procedure II was used for most experiments and in each case the drug was added at the same interval after changing the gas mixture. The results were the same whether this period was three minutes or fifteen minutes, and when this procedure was used, the hypercapnic depression was always present.

The P values indicated in the Tables containing these data (Appendix II, Tables 7 to 9), are usually less than 0.05 and support the conclusion that a hypercapnic depression occurs. Although it may appear that the S.E. values are too large to permit the small P values obtained, it should be realized that the statistical test employed took into account the fact that the values are paired. In addition, there is an obvious trend in each one of the Tables to support the existence of the hypercapnic depression.

In a bicarbonate-free bathing solution, a low pH depression of the response almost always occurred whether Procedure I or II was used. The depression, however, was not usually as great as the depression in Kreb's bicarbonate solution when 30% CO_2 was used to aerate the tissues, following Procedure II, nor was it always present at the beginning of

the series of noradrenaline doses. In many cases, there is the suggestion of a depression as the four highest dose levels of noradrenaline (5×10^{-7} to 1×10^{-5} gm/ml) only.

Figure 4A is a graph of the results obtained in a normal Kreb's bicarbonate solution when the responses are compared with the tissues being bathed in a solution aerated with 95%O_2- 5%CO_2 and with 70%O_2-30% CO_2 using Procedure II. Figure 4B is a summary of the results obtained in a normal bicarbonate-free solution when the responses are compared with the tissues being bathed in high and low pH solutions. The corresponding log-dose response curves and their linear transformations are shown in Figure 4C and 4D. The corresponding data are found in Appendix II, Tables 7A and 10A.

Figure 5 is a group of graphs chosen from the series in which the pH of the bathing media was varied in the absence of bicarbonate. The corresponding data is found in Appendix II, Tables 12B, 14A, 13C and 14C. Tables 10 to 14 contain a summary of the data obtained from all the experiments in which the noradrenaline responses were compared in High and Low pH, Bicarbonate-Free Solutions.

As the calcium ion concentration of the Kreb's bicarbonate medium was progressively reduced, the hypercapnic depression of the responses of the aorta loops to noradrenaline became greater and was not overcome by increasing the dose of noradrenaline. The summary of the data from a number of experiments, each one performed with a different concentration of calcium in the bath, is presented in Tables 7B, 7C, 8A and 8B (Appendix 2). The corresponding graphs are shown in Figure 6. The linear transformations of the dose-response curves are shown in Figure 7.

As the potassium ion concentration of the Kreb's bicarbonate medium was progressively reduced, the hypercapnic depression of the tissue responses to noradrenaline persisted relatively unchanged until the potassium ion concentration was reduced to 1/16 normal. The summary of the data is presented in Appendix II, Tables 8C, 9A, B and C. The corresponding graphs are in Figure 8.

Bicarbonate ions appear to be necessary for maximum contractility of the vascular smooth muscle. The average maximum tension attained by the tissues in a bicarbonate-free bathing medium was 1.51 grams, as compared to 1.87 grams in the Kreb's bicarbonate medium. The corresponding data is found in Appendix II, Table 22.

Figure 2

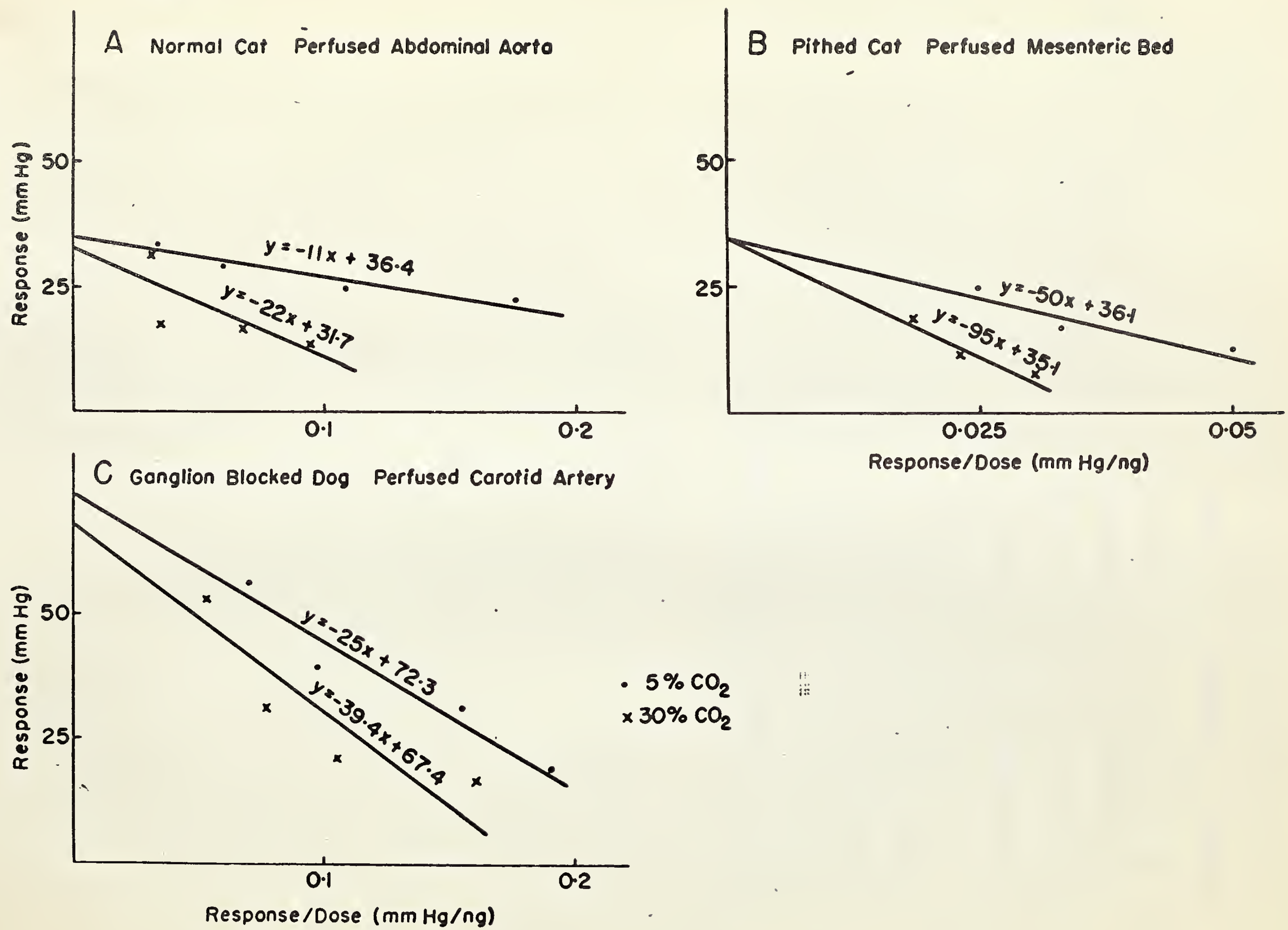

A Normal Cat Perfused Abdominal Aorta
y = -11x + 36·4
y = -22x + 31·7
Response (mm Hg)
50
25
0·1
0·2
B Pithed Cat Perfused Mesenteric Bed
y = -50x + 36·1
y = -95x + 35·1
50
25
0·025
0·05
Response/Dose (mm Hg/ng)
C Ganglion Blocked Dog Perfused Carotid Artery
y = -25x + 72·3
y = -39·4x + 67·4
Response (mm Hg)
50
25
0·1
0·2
Response/Dose (mm Hg/ng)
• 5% CO2
x 30% CO2

Figure 2

Noradrenaline Dose-Response Relationships in

Perfused Vascular Beds of Whole Animals

The corresponding data is found in Appendix II, Table 1. Linear transformations were made using the method of Mounter and Turner (1963).

Section 2. Ionic Requirements for Contractility of Aorta

Calcium

As the calcium ion concentration of the medium bathing the aorta is increased, the response to the noradrenaline increases until a maximum level of contraction is attained. The summary of the data is presented in Appendix II, Table 20, and is represented graphically in Figure 9A.

Potassium

As the potassium ion concentration of the medium bathing the aorta is increased, the response to the noradrenaline increases until a maximum level of contraction is attained. The summary of the data is presented in Appendix II, Table 21, and graphically in Figure 9B.

If the tissues were allowed to remain in a low-potassium bathing solution for longer than about a fifteen-minute period, the length of time required for relaxation after a drug-induced contraction became progressively longer. After about a forty-five minute exposure to the low potassium solution, the tissues failed to relax, even after a subsequent thirty-minute exposure to the normal solution.

Sodium

Sucrose and choline chloride can partly replace sodium chloride in a Kreb's solution without preventing aorta contractions in response to noradrenaline. When lithium chloride is used to replace sodium chloride, however, the contractions are depressed a great deal. A summary of the data in which sucrose and lithium chloride were used as

NaCl substitutes is presented in Appendix II, Tables 15 and 16. Several of the corresponding graphs are shown in Figure 10.

The aorta loops contracted more slowly in response to hypertonic Kreb's solution. The contractility in response to noradrenaline was greatly depressed when the sodium chloride content was raised to 1.5 times its normal level. When the sodium chloride concentration was doubled, the responses were almost completely absent. The loss of contractility occurs also when the osmotic pressure is raised to a similar level with sucrose, instead of sodium chloride. The effect of a hypertonic solution is at least partially reversible.

A summary of the data comparing responses in normal and hypertonic solutions is presented in Appendix II, Table 17. The corresponding graphs are shown in Figures 11A, B and C. In Table 17B which is a summary of the data comparing responses in a normal solution and a solution made hypertonic with sucrose, the depression is large and the P values confirm statistically the conclusion that contractility is depressed in a hypertonic solution. In Table 17C, which is a summary of the data comparing responses in normal solution and solutions made hypertonic with both NaCl and sucrose, the P values are not small enough to support the data statistically. However, in each case, the depression in the hypertonic solution is obviously large and difficult to dispute.

Reducing the sodium ion concentration of the solution bathing the tissues appears to potentiate the response to noradrenaline but some sodium appears to be necessary, for maximum contractility to occur. The substitute for sodium chloride used in this case was choline chloride. The summary of the data is presented in Appendix II, Table 18A and the

corresponding graph is Figure 11D. Again, the data are supported statistically and the results consistently show a small potentiation of the noradrenaline response with 1/2 Na - 1/2 choline and a small depression with 1/4 Na - 3/4 choline in the bathing solution.

Competition with Sodium

When the contractions of the loops of smooth muscle were compared in a 1/8 Ca - 1 Na and in a 1/8 Ca - 1/2 Na bathing solution, in which the osmotic pressure had been adjusted with sucrose, the responses observed in the low sodium solution were greater than those in the solution containing a normal quantity of sodium. When the contractions were compared in 1/8 Ca - 1 Na and in 1/8 Ca - 1/4 Na bathing solutions, in which the osmotic pressure had been adjusted with sucrose, the contractions at the higher dose levels of noradrenaline were depressed in the low sodium solution. The summary of the data is presented in Appendix II, Tables 18B and 18C. The corresponding graphs are shown in Figures 12A and B. It appears that, provided there is sufficient sodium for maximum contractibility to occur, there is a competition between sodium and calcium at the cell membrane. In this case, the conclusions are supported statistically.

When the contractions of the loops were compared in a normal and in a magnesium-free solution, the responses in the magnesium-free solution were greater than those in the normal solution. In contrast, when compared in a normal and in a high-magnesium solution, the responses in the normal solution were greater. The summary of the data is presented in Appendix II, Table 19. The corresponding graphs are shown in Figure

12C and D. The statistical values calculated at individual doses do not support the conclusion of a calcium-magnesium competition at the cell membrane, but the average responses were always consistent with the view that magnesium depressed responses.

Figure 3

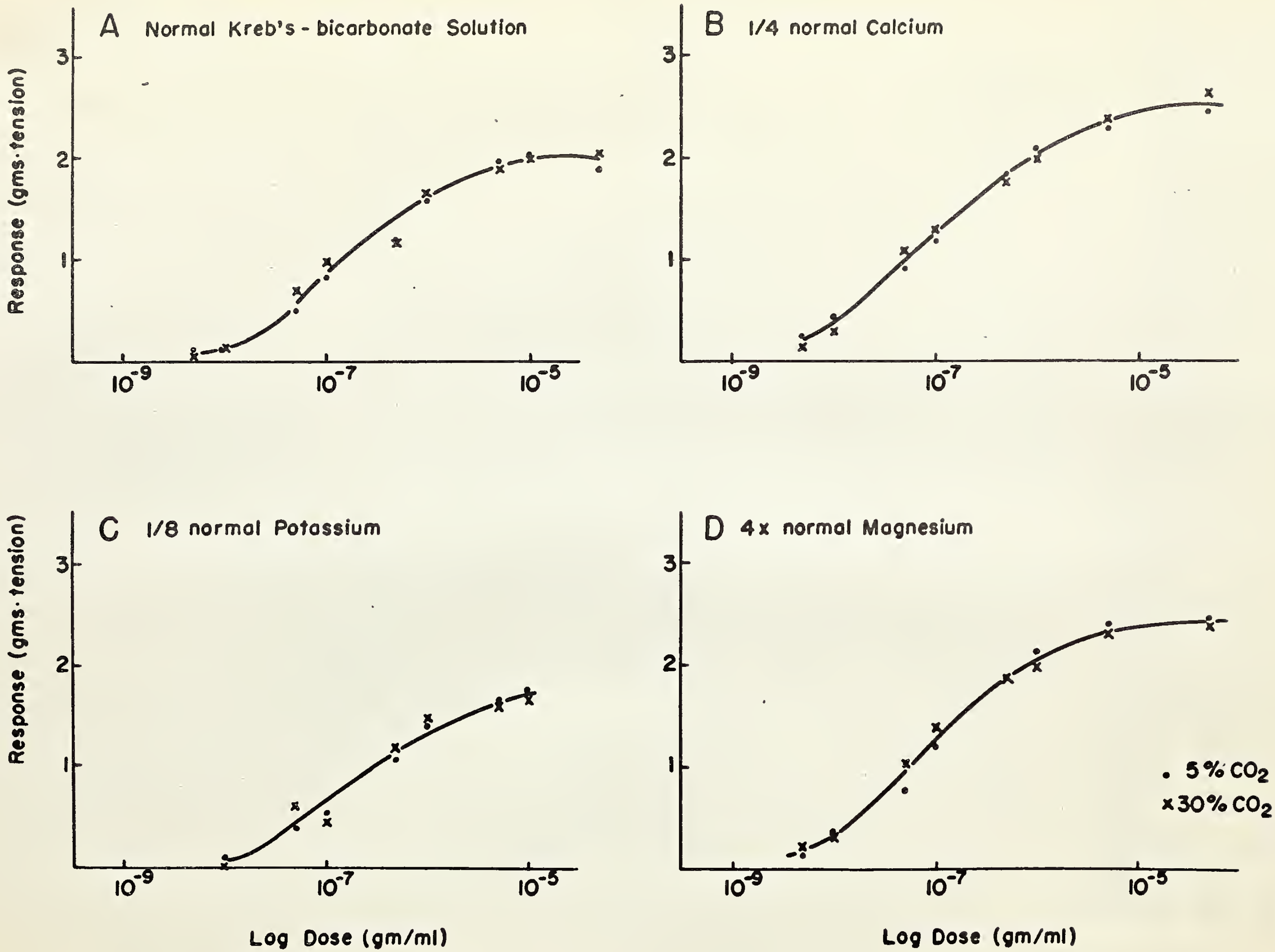

A Normal Kreb's - bicarbonate Solution
B 1/4 normal Calcium
C 1/8 normal Potassium
D 4 x normal Magnesium
Response (gms · tension)
Log Dose (gm/ml)
• 5% CO_2
× 30% CO_2

Figure 3

Noradrenaline Log-Dose Response Curves in Isolated Vascular Tissue on 5% CO_2 and 30% CO_2. Procedure I

A hypercapnic depression was not present in either the normal Kreb's-bicarbonate solution or in a solution in which the ionic concentrations were changed. The corresponding data is found in Appendix II, Tables 2A and C, 4B and 6A.

Figure 4

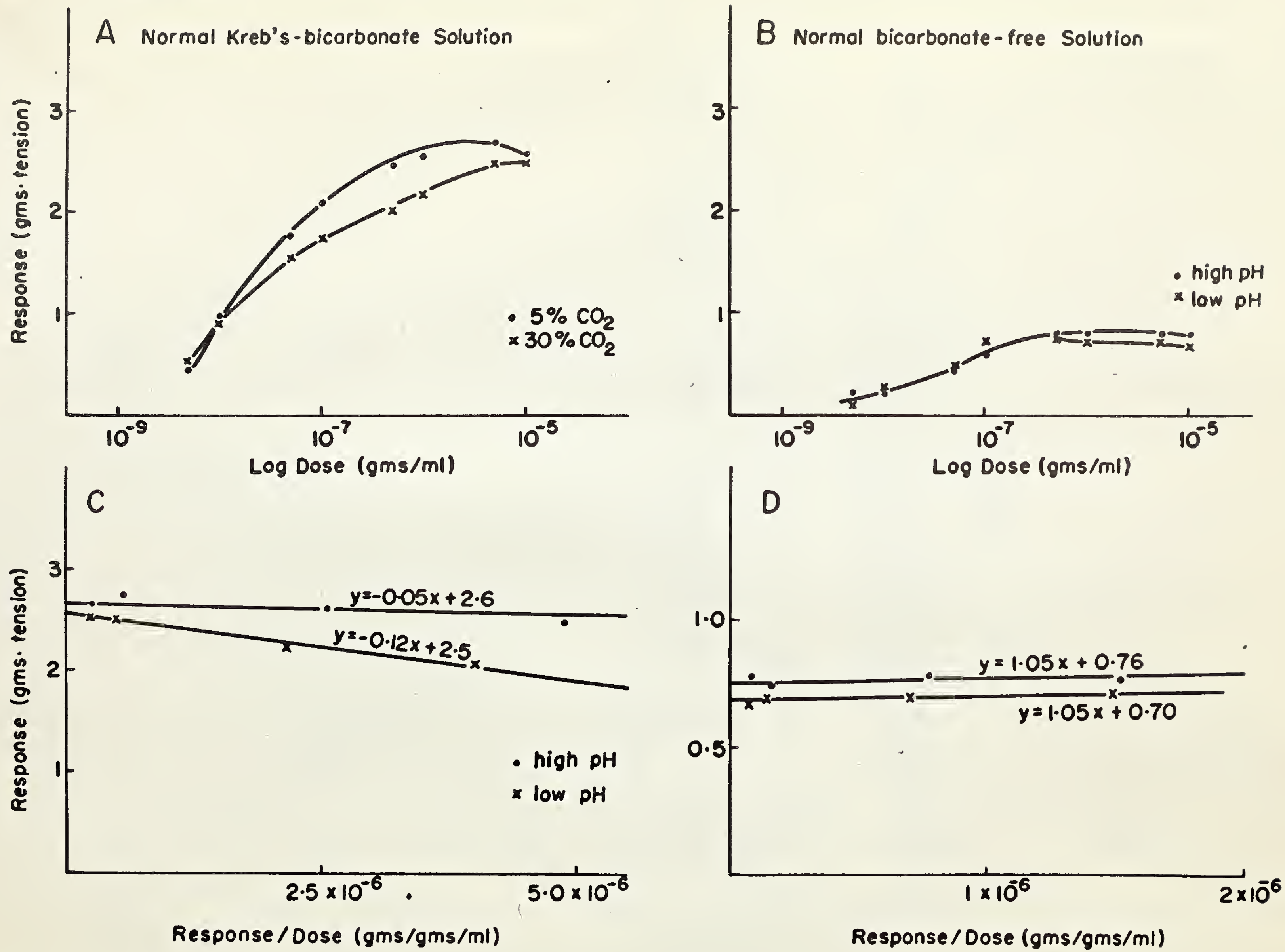

A Normal Kreb's-bicarbonate Solution
Response (gms·tension)
3
2
1
10-9
10-7
10-5
Log Dose (gms/ml)
• 5% CO2
x 30% CO2
B Normal bicarbonate-free Solution
3
2
1
• high pH
x low pH
10-9
10-7
10-5
Log Dose (gms/ml)
C
Response (gms· tension)
3
2
1
y=-0·05x + 2·6
y=-0·12x + 2·5
• high pH
x low pH
2·5 x 10-6
5·0 x 10-6
Response / Dose (gms/gms/ml)
D
1·0
0·5
y = 1·05 x + 0·76
y = 1·05 x + 0·70
1 x 10-6
2 x 10-6
Response / Dose (gms/gms/ml)

Figure 4

A, B. Noradrenaline Log-Dose Response Curves in Isolated Vascular Tissue on 5% CO_2 and 30% CO_2 (Procedure II) and in High and Low pH, Bicarbonate-Free Solutions (Procedures I and II)

A hypercapnic depression was present, but a low pH depression was not observed. The corresponding data is found in Appendix II, Tables 7A and 10A.

C, D.

Linear Transformations of the above Dose-Response Relationships.

Figure 5

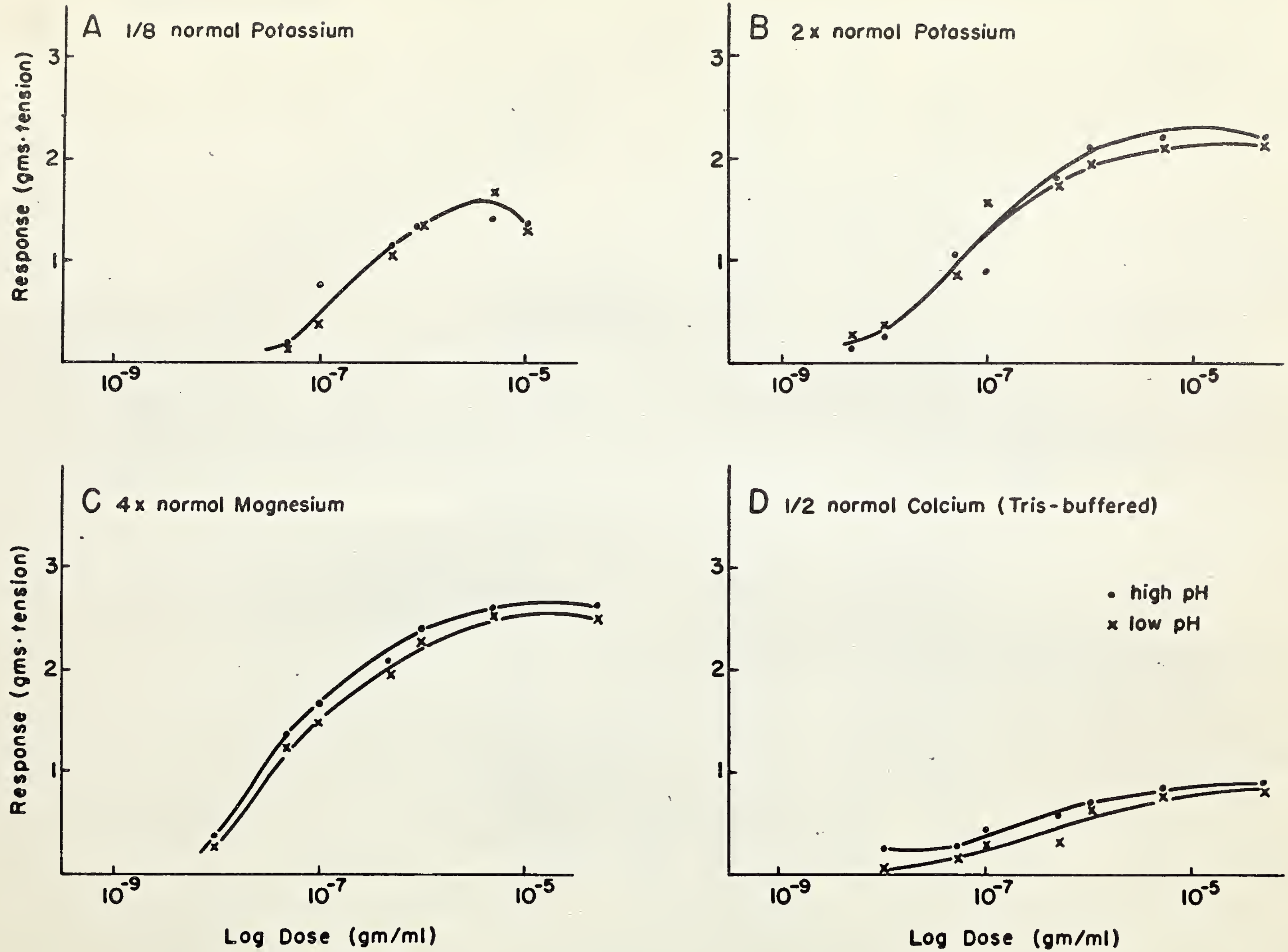
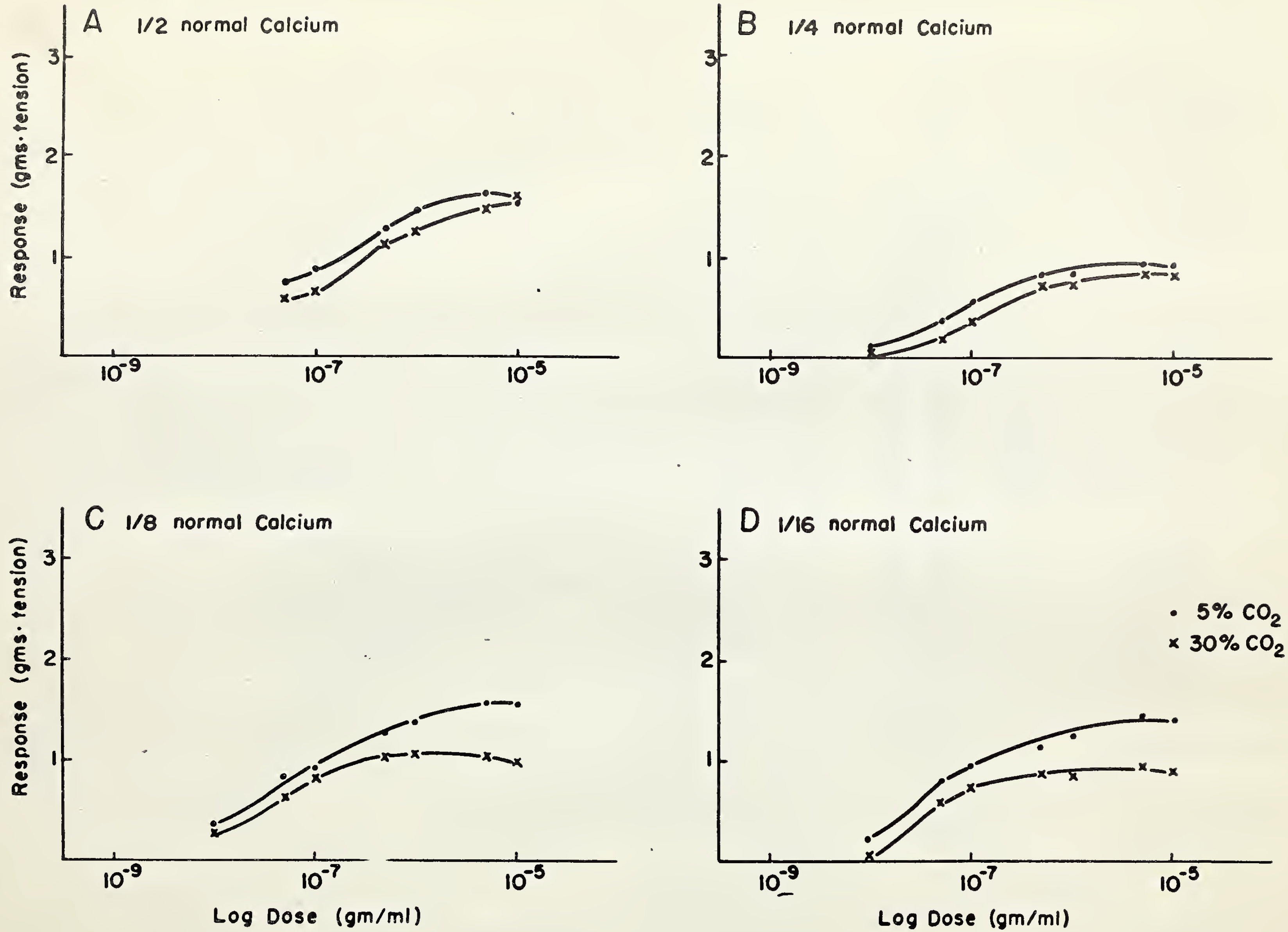

A 1/8 normal Potassium
B 2 x normal Potassium
C 4 x normal Magnesium
D 1/2 normal Calcium (Tris-buffered)
Response (gms·tension)
Log Dose (gm/ml)
10^-9
10^-7
10^-5
1
2
3
• high pH
x low pH

Figure 5

Noradrenaline Log-Dose Response Curves in Isolated Vascular Tissue in High and Low pH, Bicarbonate-Free Solutions

A low pH depression of the response to noradrenaline was almost always present, particularly at high dose levels of the drug whether Procedure I or II was used. The corresponding data is found in Appendix II, Tables 12B, 14A, 13C and 14C.

Figure 6



A 1/2 normal Calcium
B 1/4 normal Calcium
C 1/8 normal Calcium
D 1/16 normal Calcium
Response (gms·tension)
Log Dose (gm/ml)
• 5% CO_2
x 30% CO_2

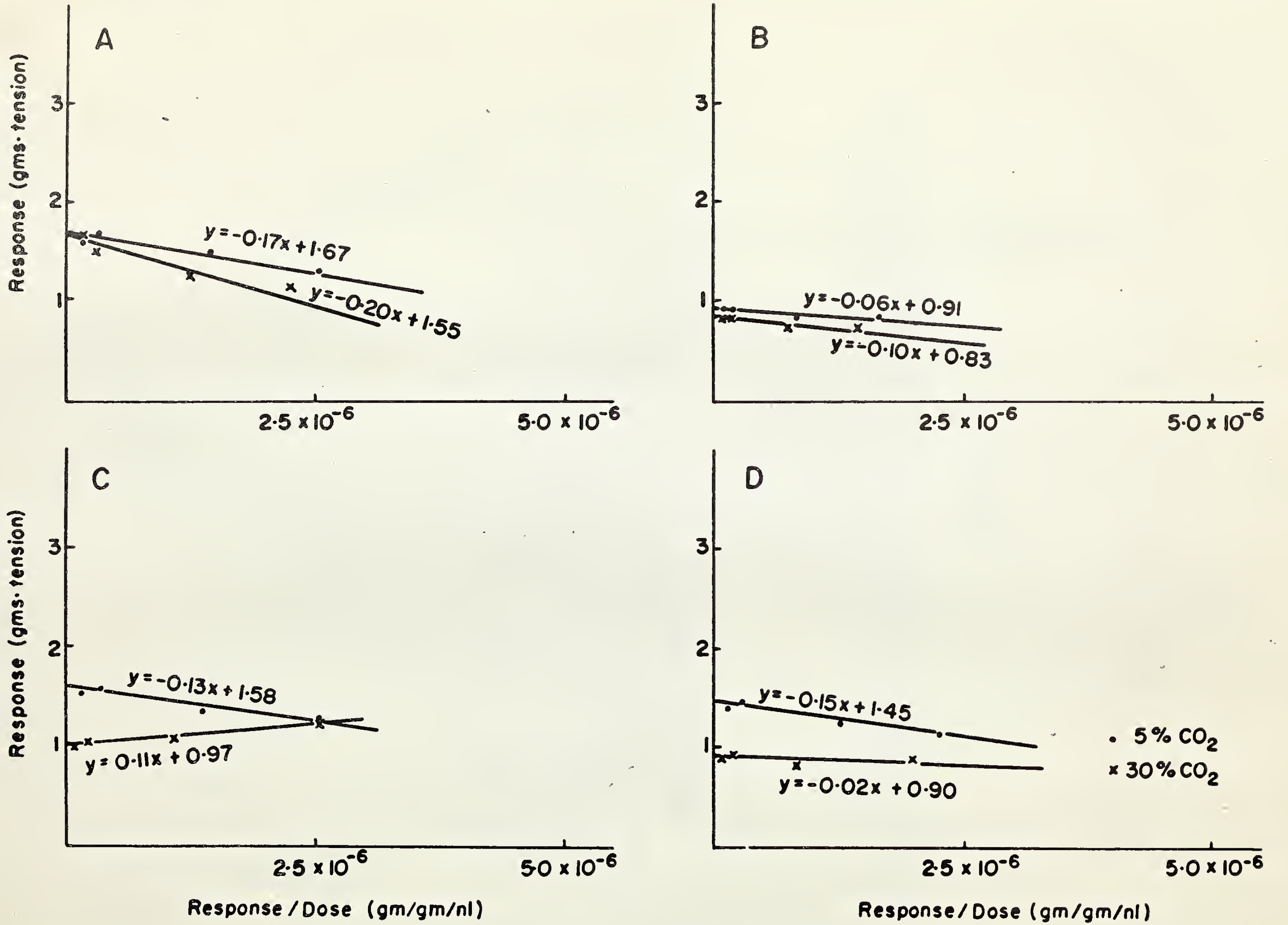
Figure 6

Noradrenaline Log-Dose Response Curves in Isolated Vascular Tissue on 5% CO_2 and 30% CO_2 with Altered Calcium Concentrations in the Bathing Media

Procedure II

As the calcium ion concentration of the Kreb's bicarbonate medium was progressively reduced, the hypercapnic depression of the aorta loops to noradrenaline became greater and was not overcome by increasing the noradrenaline dose added. The corresponding data is in Appendix II, Tables 7B and C, 8A and B.

Figure 7



A
B
C
D
Response (gms·tension)
y=-0·17x+1·67
y=-0·20x+1·55
y=-0·06x+0·91
y=-0·10x+0·83
y=-0·13x+1·58
y=0·11x+0·97
y=-0·15x+1·45
y=-0·02x+0·90
2·5 x 10^-6
5·0 x 10^-6
• 5% CO2
x 30% CO2
Response/Dose (gm/gm/nl)

Figure 7

Linear Transformations of the Noradrenaline Dose-Response Relationships of Isolated Vascular Tissue with Altered Calcium Concentrations in the Bathing Media

Corresponding data is in Appendix II, Tables 7B and C, 8A and B.

Figure 8

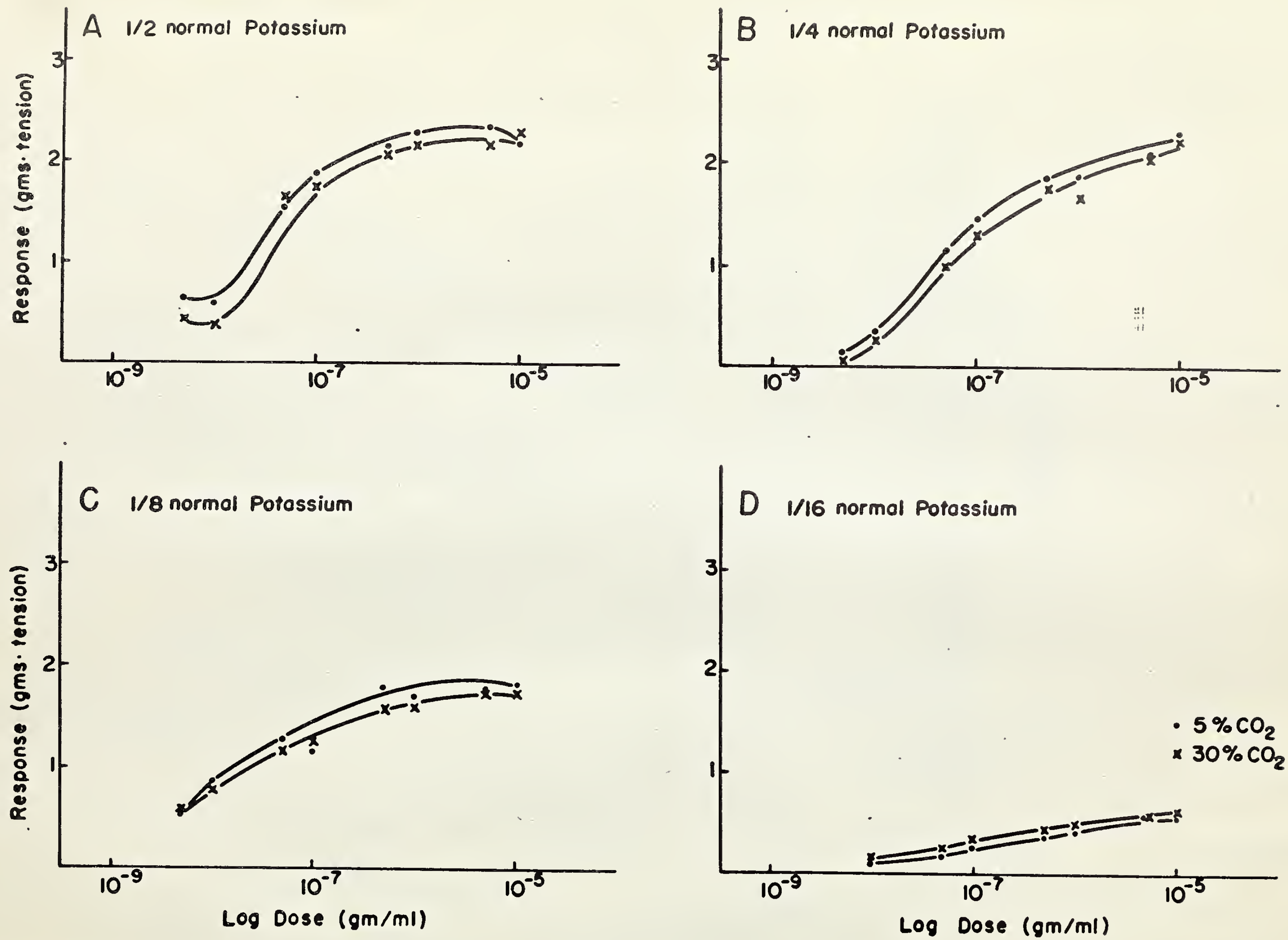
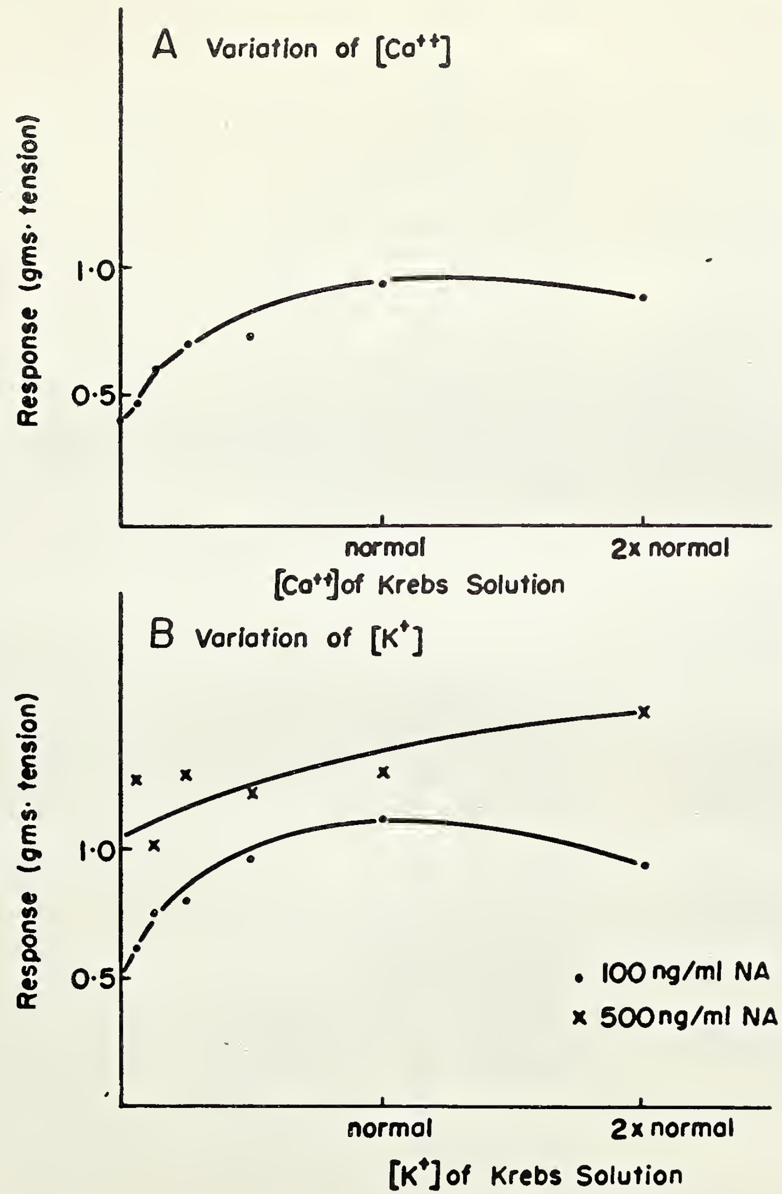

A 1/2 normal Potassium
B 1/4 normal Potassium
C 1/8 normal Potassium
D 1/16 normal Potassium
Response (gms· tension)
Log Dose (gm/ml)
10^{-9}
10^{-7}
10^{-5}
1
2
3
• 5% CO_2
x 30% CO_2

Figure 8

Noradrenaline Log-Dose Response Curves in Isolated Vascular Tissues on 5% CO_2 and 30% CO_2 with Altered Potassium Concentrations in the Bathing Media

As the potassium concentration of the Kreb's bicarbonate medium was progessively reduced, the hypercapnic depression of the tissue response to noradrenaline persisted relatively unchanged until the potassium ion concentration was reduced to 1/16 normal when a small but consistent hypercapnic potentiation occurred. The corresponding data is found in Appendix II, Tables 8C, 9A, B and C.

Figure 9



A Variation of [Ca++]
Response (gms· tension)
1·0
0·5
normal
2x normal
[Ca++] of Krebs Solution
B Variation of [K+]
Response (gms· tension)
1·0
0·5
• 100 ng/ml NA
x 500 ng/ml NA
normal
2x normal
[K+] of Krebs Solution

Figure 9

A.

Relationship Between External Calcium Ion Concentration and Vascular Tissue Contractility

As the calcium concentration of the medium bathing the aorta loops is increased, the response to the noradrenaline increases until a maximum level of contractility is attained.

B.

Relationship Between External Potassium Ion Concentration and Vascular Tissue Contractility

As the potassium concentration of the medium bathing the aorta loops is increased, the responses to 100 ng doses of noradrenaline increases until a maximum level of contraction is attained. When 500 ng doses of noradrenaline were used the responses continued to increase with the concentration of potassium.

Figure 10

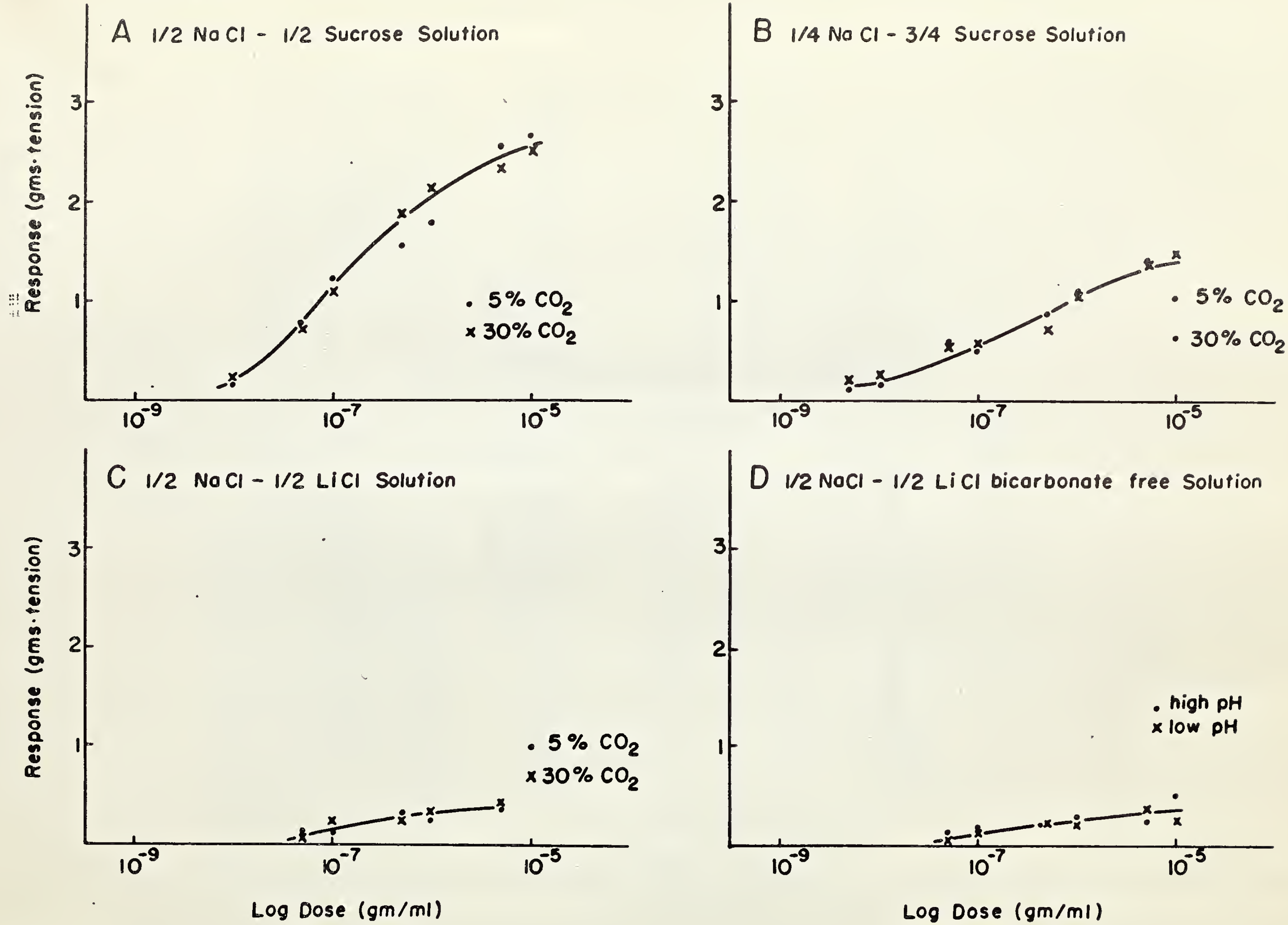

A 1/2 Na Cl - 1/2 Sucrose Solution
B 1/4 Na Cl - 3/4 Sucrose Solution
C 1/2 Na Cl - 1/2 LiCl Solution
D 1/2 NaCl - 1/2 Li Cl bicarbonate free Solution
Response (gms·tension)
Log Dose (gm/ml)
• 5% CO2
x 30% CO2
• 30% CO2
• high pH
x low pH
1
2
3
10-9
10-7
10-5

Figure 10

Noradrenaline Log-Dose Response Curves for Isolated Vascular Tissue on 5% CO_2 and 30% CO_2 with Altered Sodium Concentrations in the Bathing Media

Sucrose replaced sodium chloride in a Kreb's solution without preventing aorta contractions in response to noradrenaline. When lithuim chloride was used to replace sodium chloride, the contractions were greatly depressed. The corresponding data is found in Appendix II, Tables 15A, C and B and 16B.

Figure 11

Variation of Sodium Concentration and Osmotic Pressure

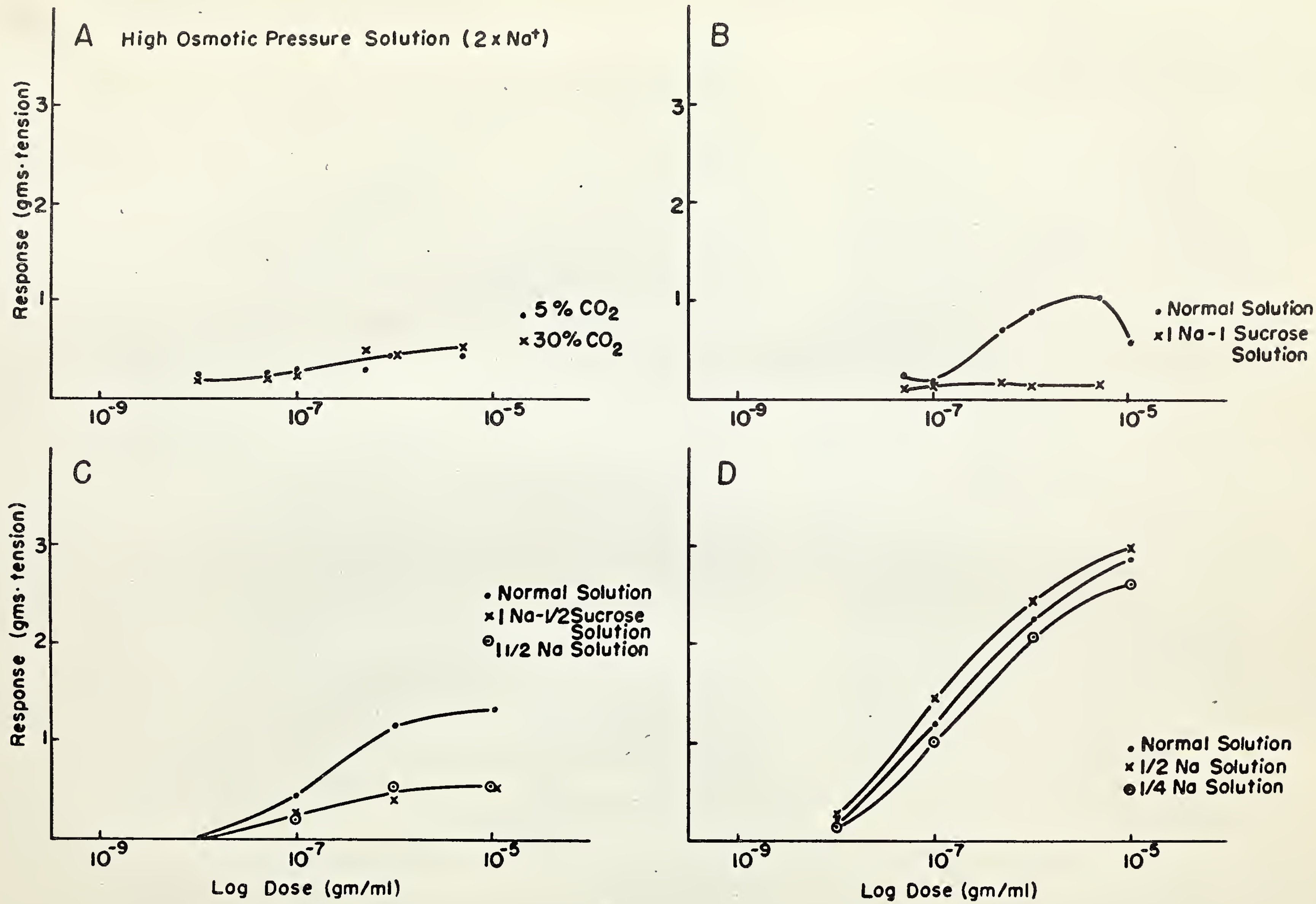


Figure 11

Noradrenaline Log-Dose Response Curves in Isolated Vascular Tissue with Altered Sodium Concentrations and Osmotic Pressure in the Bathing Media

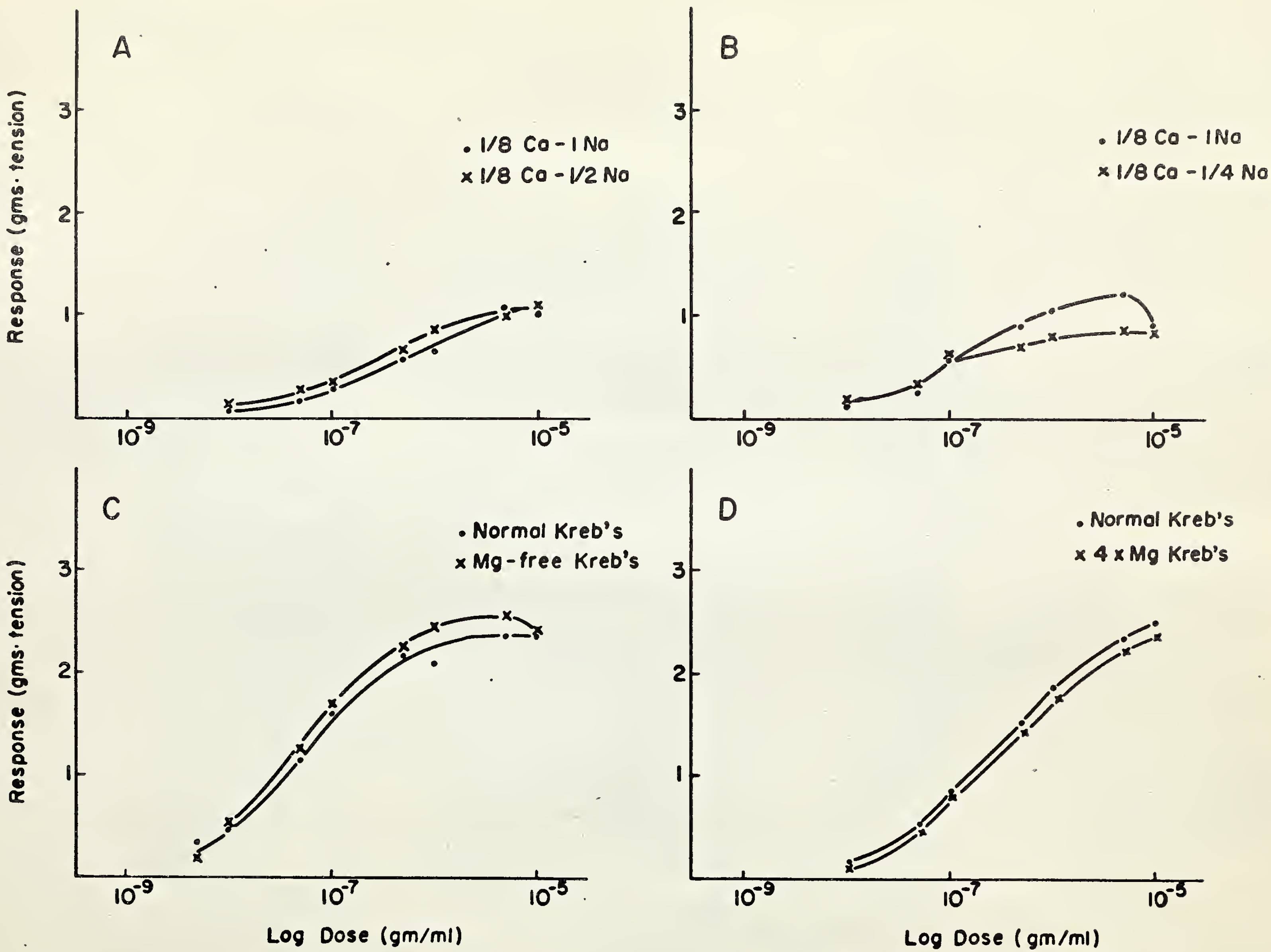
A, B, C.

The contractility of the aorta loops in response to noradrenaline was depressed when the sodium chloride concentration of the Kreb's solution was raised to 1.5 times its normal level. When the sodium chloride concentration was doubled, the responses were almost completely absent. A similar loss of contractility occurred when the osmotic pressure was raised to the same level with sucrose instead of sodium chloride. The corresponding data is found in Appendix II, Tables 17A, B and C.

D.

Reduction of the sodium concentration of the Kreb's solution to half normal appeared to potentiate the response to noradrenaline. A further decrease to one-quarter normal then depressed the response. The corresponding data is found in Appendix II, Table 18A.

Figure 12



A
B
C
D
Response (gms·tension)
3
2
1
10-9
10-7
10-5
• 1/8 Ca - 1 Na
x 1/8 Ca - 1/2 Na
• 1/8 Ca - 1 Na
x 1/8 Ca - 1/4 Na
• Normal Kreb's
x Mg-free Kreb's
• Normal Kreb's
x 4 x Mg Kreb's
Log Dose (gm/ml)

Figure 12

Noradrenaline Log-Dose Response Curves in Isolated Vascular Tissue with Altered Sodium Concentrations and Magnesium Concentrations

A, B.

The contractions of the aorta loops in a 1/8 Ca - 1/2 Na bathing solution were greater than those in a 1/8 Ca - 1 Na solution, while those in a 1/8 Ca - 1/4 Na bathing solution were smaller. The corresponding data is found in Appendix II, Tables 18B and C.

C, D.

The contraction of the aorta loops in a Mg-free bathing solution were greater than those in a normal solution, while those in a 4 x normal Mg bathing solution were smaller. The corresponding data is found in Appendix II, Tables 19A and B.

DISCUSSION

Carbon Dioxide and pH Effects

In the first series of experiments, the existence of a hypercapnic depression of the noradrenaline response in arterial smooth muscle of the whole animal was supported. Shivak, who had previously done similar work in this laboratory, plotted his results, using the linear transformation of the sigmoid curve, which had been proposed by Lineweaver and Burke. According to these plots, the maximum blood pressure response appeared to be the same whether the animals were respired with 5% CO_2 or 30% CO_2. The hypercapnic depression then can be overcome with sufficiently high doses of noradrenaline. The sigmoid curves from the current series of experiments were converted into linear plots, using the transformation proposed by Eadie (Mounter, Turner, 1963). These plots, just as those of Lineweaver and Burke, indicate that the maximum blood pressure response which can be attained tends to be the same with both a high and a low concentration of carbon dioxide. This suggests the possibility that the carbon dioxide may exert its effect in a competitive manner.

It was hoped that the mechanism of the carbon dioxide inhibition might be worked out by using isolated pieces of aorta in an organ bath. Two procedures were used to obtain a noradrenaline log dose-response curve (see methods). When the first procedure was used, before noradrenaline administration, CO_2 acted on some tissues for a longer period of time

than it did on others, and the hypercapnic depression of the vascular smooth muscle could not be demonstrated. When the second procedure was used, the tissues were exposed to each level of carbon dioxide for approximately the same length of time (about 10 minutes) before noradrenaline was added. Under these experimental conditions a hypercapnic depression occurred.

With both experimental procedures, a low pH depression was usually observed in the bicarbonate-free solutions, particularly at the higher dose levels of noradrenaline. The depression in the low pH solutions tended to be less than that in solutions in which the pH was lowered with carbon dioxide. Nash has suggested that this smaller depression might be explained by the fact that carbon dioxide diffuses into membranes more readily than H^+ ions (Nash, 1962). Other workers, using spiral strips of rat aorta, reported that, when respiratory acidosis was simulated with 30% CO_2, the depression of the noradrenaline response was much greater than that when metabolic acidosis was simulated with altered concentrations of $NaHCO_3$. They also suggested that the explanation might be found in the fact that carbon dioxide has considerable solubility in non-polar fat solvents, whereas the H ion has not (Tobian et al., 1959). There is the possibility that CO_2 may diffuse into an ion exchange membrane with fixed charges more readily than does the H_3O^+ ion. This would mean then that the depression is effected through the cellular membrane.

The depression or lack of it under different experimental procedures might be explained by considering the length of time during which the tissue was exposed to 30% CO_2. The experimental results indicated

that the CO_2 effect in isolated rabbit aorta strips occurs quickly because a three-minute exposure to high CO_2 is a sufficiently long time for the depressant effect to appear. It persists for at least fifteen minutes. Probably there is a tendency for the high CO_2 effect to be compensated during a sufficiently long exposure, so that the depression is gradually overcome. Perhaps the critical factor in determining the level of contracture in response to a stimulus then is the H_i/H_o ratio across the membrane.

The log dose-response curves indicate that decreasing the calcium ion concentration of the medium bathing the loops of aorta produces a larger hypercapnic depression of the noradrenaline response, particularly at high dose levels of the drug. The linear transformations of the data suggest that in a normal Kreb's bicarbonate bathing medium, the hypercapnic depression can be overcome by sufficiently high doses of noradrenaline, so that the maximum response is the same with both 5% and 30% CO_2. This competitive type of effect is not present at the lower concentrations of external calcium and the transformed curves suggest that, with low external calcium concentrations, the hypercapnic depressant effect is greater at high doses of noradrenaline.

The concept of Woolley, who attempts to explain the contraction of smooth muscle in response to serotonin, may be a help in understanding the mechanism of the hypercapnic depression. Woolley postulates the contraction of smooth muscle to be due to a reaction between Ca^{++} ions, actomyosin and ATP and that serotonin combines with a lipid receptor substance which aids the passage of Ca^{++} ions across the membrane to initiate a

muscle contraction (Woolley, 1958). In at least partial support of Woolley's theory, workers have reported that the effectiveness of drugs on smooth muscle is due to the fact that a drug increases membrane permeability to calcium (Durbin, Jenkinson, 1961; Evans, Thesleff, Schild, 1958). The quick recovery of smooth muscle preparations after the addition of calcium to a calcium-free medium suggests that the calcium effect is an extracellular one and that the slow onset of the effects due to calcium loss are due to the slow diffusion outwards of calcium (Robertson, 1960). This, however, is probably not correct. Analysis of the pertinent diffusion equations suggests that the marked differences in the loss of contractility in Ca and in Sr Ringer's solutions required postulation of differential binding of calcium and strontium at the cell surface and the release of calcium and strontium from binding by drugs initiating contraction. Contraction would result because the activity of calcium and strontium in the membrane would be increased and because the unbinding also increases the ability of these ions to penetrate the membrane (Daniel, 1963).

It is necessary to postulate a mechanism of action of CO_2 whereby the activity of the cell is decreased. $R\text{-}CA + NA \rightleftharpoons R^{=} + Ca^{++} + NA$. The addition of the pressor agent to the medium bathing the smooth muscle cell causes the release of calcium from its binding sites in the membrane. The calcium ions which are liberated into the intracellular space are responsible for the contraction of the smooth muscle cell. The more noradrenaline is added, the more calcium is released intracellularly and the greater the resultant contractile response up to a maximum level of contractility.

To explain the depression of hypercapnia, one can postulate a greater affinity of calcium for its membrane binding sites as a result of the action of carbon dioxide. This could be the result of two distinct mechanisms: 1. The CO_2 may alter the affinity of receptor sites for the calcium. 2. CO_2 may affect the hydration of the ions at the lipid-water interface as the cell membrane, thereby releasing sodium from its water of hydration (Sears, Eisenberg, 1961). The heat of hydration of calcium is much higher than that of sodium and potassium and it is plausible to attribute the much lower permeability of calcium in the membrane to the much less frequent escape of singly hydrated calcium ions from the water structure (Mullins, 1960). If one assumes that sodium and possibly potassium compete with calcium at the membrane, (Briggs, Melvin, 1961), the decrease in the activity of these competing ions favours the surface binding of the calcium at the membrane in the presence of carbon dioxide.

Sears and Eisenberg have observed the effects of CO_2 at a cell membrane model consisting of water and oil (Benzene) phases. In this model, they measured the interfacial tensions between the two phases and calculated the effects of electrolyte solutions and of CO_2 on the molecular arrangements at the interface. They found that bicarbonate ions, unlike chloride ions, caused a marked decrease in interfacial tension. Concomitant with this decrease in interfacial tension was an increase in hydration of the interface and changes in molecular spacings of the lipid. This hydration may be considered as reflecting a more ionic-permeable cell membrane. The addition of the CO_2 to the bicarbonate containing salt solution caused an increase in interfacial tension of the model approaching that of the chlorides with decreased hydration of the interface.

According to Sears and Eisenberg, if this is viewed as occuring at the cell membrane, this would make the lipid more continuous and decrease the ease of ionic penetration (Sears, Eisenberg, 1960). Conceivably, calcium tightly bound to anionic sites would be less affected by the decreased hydration at the interface than the other cations because calcium escapes from hydration by a more complicated stepwise process of shedding hydration than that for singly charged ions (Mullins, 1960).

At high dose levels of noradrenaline, the hypercapnic depression of the response is overcome, because the excess noradrenaline which is necessarily present can compensate for its decreased effectiveness in releasing calcium. As the calcium ion concentration of the bathing medium is decreased, it is probable that the amount of calcium bound at membrane sites is decreased. In addition to a decreased level of calcium, there is an altered calcium gradient between the membrane and the extracellular fluid which is due to the removal of the normally high level of calcium in the extracellular fluid. Consequently, for any dose level of noradrenaline, less membrane calcium is available to be released intracellularly and even this amount is now released into both the extracellular and intracellular fluids. This decreases the maximum level of tension which it is possible for the tissues to attain in contraction at a given concentration of noradrenaline. The CO_2 exerts its typical depressant effect, but this cannot be overcome by high dose levels of the pressor drug.

The low pH depression of the noradrenaline response in bicarbonate-free solutions is more consistent, although smaller than the hypercapnic depression. It is conceivable that a low pH of the bathing medium de-

presses contractility by increasing the affinity of calcium for its receptors at the cell surface in much the same way the CO_2 does. The further increase in binding which the CO_2 induces due to a decrease in the hydration of the interface would not occur in this case, so that the depression caused by a low pH in the absence of bicarbonate is smaller than that caused by CO_2.

Bicarbonate ions are necessary for maximal contractions of muscle to occur. It has been shown that, when bicarbonate is removed from the fluid bathing the rat diaphragm, the contractions of the diaphragm in response to electrical stimulation are reduced in amplitude, even when there is no simultaneous pH change (Creese, 1950). This can be implied from this series of experiments, because the maximum tensions which the tissues attain in contraction in a bicarbonate-free solution are, on the average, lower than those in a normal Kreb's bicarbonate solution (1.44 and 1.82 grams tension, respectively).

When changing his solution from a bicarbonate solution to a bicarbonate-free solution, Creese observed that there was initially a rise in contraction amplitude, then the fall. When the switch was made in the other direction, there was a temporary decrease in contraction amplitude. This was attributed to the initial effect of a hypercapnic depression before it was overcome by the bicarbonate effect because the Kreb's bicarbonate solution was respired with 95%O_2-5%CO_2, while the other solution was respired with 100% O_2.

Woolley's theory of the action of serotonin and the theory of the mechanism of the inhibition caused by low pH and CO_2, described above, can be applied to many pressor agents. Ariens reported an in-

creased response of guinea pig ileum to histamine as the pH increased (pH 6.8→7.1→8.3). Other workers have reported that $H_2CO_3(CO_2)$ inhibits the stimulating action of histamine on guinea pig intestine and of oxytocin on the uterus, although the action of other stimulating agents including adrenaline was not affected. A similar pH change induced with sodium maleate and maleic acid was ineffective in producing a depression (Halpern et al., 1959).

Potassium ions are also implicated in the contraction of smooth muscle in response to catecholamines. During the infusion of acids into the dog, the extracellular fluid potassium ion concentration rises (Brown, Goott, 1963; Young et al., 1960). In smooth muscle, the contractile action of adrenaline and noradrenaline is accompanied by a selective increase in the outflow of potassium from smooth muscle (Daniel, Dawkins, Hunt, 1957).

A proposed explanation of the potassium shift is that it occurs in response to factors tending to keep the H_i/H_o ratio equal to K_i/K_o. During both respiratory and metabolic acidosis, H_i/H_o falls, so that the potassium extrusion into the extracellular fluid is expected (Brown, Goott, 1963). In these experiments, the effect of hypercapnia on the noradrenaline response of the aorta appeared to act similarly at all levels of K_o in the bath, until the 1/16 normal level of K was reached. At this level, instead of the expected hypercapnic depression, there was consistently a slight potentiation of the response.

The failure of the hypercapnic depression in the 1/16 K solution could result if the action of low potassium is the same as that of a high CO_2 concentration, that is, if decreasing $[K^+]$ results in an in-

creased affinity of the binding site for calcium. This effect would be expected if potassium normally competes with calcium for membrane binding sites. Partial removal of potassium would increase the amount of calcium bound at the cellular membrane and, therefore, if the effect of the CO_2 is to increase calcium binding it would be ineffective in a low potassium solution where calcium binding was already maximal.

Ions and Arterial Smooth Muscle Contractility

Barr has outlined the three steps involved in smooth muscle contraction as follows:

(a) Change in membrane permeability.

(b) Coupling event and an increase in free intracellular calcium ion.

(c) Contractile event - phosphate bond energy is transformed into mechanical energy (Barr, 1961).

The contractions of the aorta in response to one dose level of noradrenaline were compared in solutions of varying calcium content. The results indicate that an increase in external calcium produces an increase in the contractile response. It was expected that the contractility in the calcium-free medium would become negligible, (Daniel, et al., 1962). However, even after bathing in such a medium for over three hours, a residual response which was about 45% of the maximum response was still present (Figure 9A). Since the Ca-free solution was changed at least every ten minutes, residual response might be attributed to the presence of calcium containing impurities in the laboratory reagents used to mix the solutions. It might also be due to the ability of the tissue to use bound membrane calcium to produce a contractile response.

Calcium appears to exert its stimulating action in excitable tissue by entering into cells when they receive a stimulus (Briggs, Melvin, 1961; Durbin, Jenkinson, 1961). Hinke and Wilson measured the contractility of an isolated arterial segment by measuring fluid flow at a constant perfusing pressure through the lumen of the vessel. K-contracture increased in proportion to the external calcium ion concentration while drug contraction appeared as a near maximal response between 0.5 and 0.75 mM/litre of calcium. This led them to conclude that the calcium which enters during K-depolarization comes from calcium in the extracellular fluid, while the calcium which enters on drug excitation is released from bound membrane calcium (Hinke, Wilson, 1963). If this interpretation is correct, it might be used to explain the residual response to noradrenaline in the calcium-free medium in the experiment described above. However, unlike the results in the experiments of Hinke and Wilson, the results in these experiments show a continuously increasing response to noradrenaline as the external calcium concentration increases to a 2.5 mM concentration.

Potassium is also implicated in the contraction of smooth muscle. When the tissues were allowed to remain in a low potassium bathing medium for more than about thirty minutes, they failed to relax following a contraction in response to noradrenaline. While the experiments were in progress, the tissues were allowed to remain in the low potassium solution for ten minutes only before the drug was added. They were allowed to relax in a normal Kreb's bicarbonate solution. When the experiments were performed in this way, it was assumed that there was not sufficient time for the K_i content of the arterial tissues to be changed significantly

while they were in a solution with an abnormal potassium content. Therefore, the results observed could be attributed to either the altered K_o value or to the altered K_i/K_o ratio.

It was found that the response to a particular dose level of noradrenaline increased as the potassium ion concentration of the bathing medium increased up to a maximum in normal Kreb's bicarbonate solution. As the potassium ion concentration was raised to still a higher value, the response began to decrease again (Figure 9B). The finding that contractility increases as K_o increases is a common one (Bohr, Goulet, 1961; Brodie, Brodie, Cheu, 1957).

Barr _et al._ found that, in addition to a high K_o contraction, a low K_o contraction develops slowly in arterial smooth muscle. This contraction was observed particularly when the tissues were placed in a K-free solution. It was preceded by a five to ten minute latent period which suggested that the contracture is due to a decrease in K_i. When even a small amount of K (0.5 and 1.5 mM) was present, the onset of the contracture was delayed and its magnitude was not as great (Barr _et al._, 1963). In the present experiments, the low K_o contracture was not observed, even in the few tissues which were kept in a 0.75 mM (1/8 normal) solution for more than thirty minutes.

Potassium efflux is associated with smooth muscle stimulation (Barr, 1961). Barr _et al._ have summarized the altered K effects as follows:

(1) Increased K_i increases contractility.

(2) Increased K_i increases relaxation rate.

(3) Excitability is decreased by too high or too low a K_i/K_o ratio. --and

(4) The extent of tonic shortening depends on the K_i/K_o ratio (Barr et al., 1963).

Sodium does not appear to be as important a cation in the adrenergic response of arterial smooth muscle as its high concentration in the extracellular fluid might suggest. It was found that 75% of the sodium chloride in the Kreb's medium could be replaced with sucrose or choline chloride without abolishing the contractile response of the aorta loops to noradrenaline (Figure 10B).

Dose-response curves to noradrenaline were compared in normal, 1/2 normal and 1/4 normal Na Kreb's bicarbonate solutions. The contractility was increased in the 1/2 Na solution but decreased in the 1/4 Na solution, as compared to the contractions in a normal solution (Figure 11D). It appears that a decrease of Na_o has a potentiating effect until a crucial concentration is reached beyond which the effect becomes one of depression.

A number of persons have reported a potentiation of the pressor response in smooth muscle when $[Na^+]_o$ is decreased (Bohr, Brodie, 1958; Brodie, Brodie, Cheu, 1957; Williamson, Moore, 1960). In some cases, this has been attributed to an osmotic pressure effect (Williamson, Moore, 1960; Hinke, Wilson, 1962). Others, however, have reported that there are no variations in the amplitude of contraction of rabbit aorta strips in response to adrenaline, if only osmotic pressure changes with sucrose occur (Brodie, Brodie, Cheu, 1957). In support of the data dealing with low Na_o potentiation of the contraction, it has been reported that adrenaline increases Ca^{45} efflux 105% in normal aortas and 225% in aortas in which the sodium chloride concentration of the bathing

medium is lowered (Briggs, Melvin, 1961). Other workers have found that a reduction of the concentration of sodium chloride in the bathing medium diminished smooth muscle contractility. However, these persons decreased the concentration of the NaCl to less than half normal so that their results also conform to those reported here (Dodd, Daniel, 1960).

Doubling the Na_o of the bathing medium caused a large depression of the tissue response to noradrenaline, so that almost no contraction occurred in response to the drug. This effect appears to occur as a result of the corresponding increase in osmotic pressure, because the depression of contraction, when the osmotic pressure was raised with sucrose, was similar to the high sodium depression (Figures 11B and 11C). As soon as the tissues were placed in contact with the high osmotic pressure solutions, a slow contracture of the tissue began to occur. It is possible, in fact, that increasing the osmotic pressure to a value equal to that of a solution in which Na_o is doubled abolishes aortic contractions and that the responses recorded in such solutions are merely increases in tone due to the high osmotic pressure. The depression due to the high osmotic pressure and the simultaneous increase in the smooth muscle tension are both reversible for when a normal solution is restored to the tissues, the contractility and the basal tension are both restored to their control values.

The potentiation of the noradrenaline response when the tissues are bathed in a low sodium solution suggests the possibility of a Na-Ca competition in the cell, possibly for the receptor which binds calcium in contraction. This is supported by data obtained from an experiment in which contractions in response to noradrenaline are compared in

1/8 Ca - 1 Na and 1/8 Ca - 1/2 Na solutions (Figure 12A). However, contractions of the aorta in a 1/8 Ca - 1/4 Na solution were markedly reduced when compared to those in a 1/8 Ca - 1 Na solution (Figure 12B). This would be explained by our previous suggestion that, although there may be a competition between sodium and calcium for calcium binding sites in the arterial smooth muscle, there is also a particular level of sodium which is necessary for maximum contractility to occur.

When lithium chloride was used as a substitute for part of the sodium chloride, the contractility of the aorta was drastically depressed (Figures 10C and 10D). Unlike sucrose and choline, but like sodium, lithium ions can enter some cells upon excitation but they are transported out very inefficiently by the sodium pump (Keynes, Swan, 1959; Armett, Ritchie, 1963). Using rabbit intestinal arteries, Waugh observed that the replacement of NaCl with LiCl impaired the speed and amplitude of contraction, although there was some response even in the total absence of sodium. The vaso-depressant effects of lithium developed after an exposure of several minutes. Waugh interpreted this to suggest that lithium depressed at an intracellular location or through slow membrane displacement of a substance complexed in an undissociated manner. A possibility might be that lithium is a more effective competitor with calcium than is sodium.

A comparison of the dose-response curves to noradrenaline in normal Kreb's bicarbonate solution to those in solutions with an altered magnesium concentration suggest a calcium-magnesium competition occurring in the cell or at its membrane. The contractility of the aorta was potentiated in a magnesium-free solution (Figure 12C), and depressed in a

high magnesium solution (Figure 12D). It appears then that magnesium can replace calcium at the membrane but cannot act in an excitatory capacity.

It will be recognized that often (as in the case of variation of magnesium concentration) the statistical tests which were applied to the data, at individual doses obtained in these experiments failed to show significant differences in seeming contradiction to some of the conclusions drawn. In several cases, they did do so, but when they did not show yield significant differences, conclusions were drawn on the basis of consistent differences between responses at all or several continuous doses. To take statistical account of such consistent differences between responses at a variety of doses is a complex problem requiring the use of some procedure to fit the dose-effect curves to some non-linear function so that deviations attributable to the change in response with dose can be calculated. This did not seem a practical procedure.

SUMMARY AND CONCLUSIONS

1. The depressant effect of hypercapnia on the blood pressure response of intact animals to noradrenaline was observed. Different vascular beds of dogs and cats were perfused at a constant rate of blood flow and the effects of varying doses of noradrenaline were plotted, using a linear transformation of the log dose-response curves. Both the normal and the hypercapnic responses tended towards the same maximum value suggesting that high dose levels of noradrenaline overcome a hypercapnic depression. These data confirm earlier work done in this laboratory (Shivak, 1961).

2. Experiments were performed on isolated loops of rabbit aorta to record its contractility in response to noradrenaline. The effects of variation of pH with and without carbon dioxide and of variation of the cation concentrations in the bathing medium on this contractility were observed.

3. A hypercapnic depression of the response to noradrenaline was observed when the length of time of exposure of the tissues to 30% CO_2 gas before the addition of noradrenaline was kept fairly short (up to fifteen minutes). When the length of time of exposure to the 30% CO_2 was increased, the depression was not observed, suggesting that the depressant effect of high CO_2 is overcome with time.

4. A low pH in a bicarbonate-free solution produced a depression of the responses to high doses of noradrenaline which was not as large as the one induced by hypercapnia but was observed after both short and long

exposures to the low pH solution.

5. The presence of bicarbonate ions in the medium bathing smooth muscle cells appears to be necessary for maximum contractility of the aorta. The maximum average response of tissues in the bicarbonate-free solutions was less than that in Kreb's bicarbonate media.

6. When the ions in the solution bathing the tissue loops were present in normal concentrations, the depression due to hypercapnia or a low pH, bicarbonate-free solution appeared to be overcome by high dose levels of noradrenaline. As the external calcium ion concentration was decreased, the hypercapnic depression became greater and could no longer be overcome by high dose levels of the drug.

7. It is suggested that carbon dioxide acts at the membrane to depress the release of bound calcium into the intracellular space where it effects a contraction. This depression can be overcome by high dose levels of noradrenaline, as long as there is an excess amount of calcium present in the external bathing medium.

8. Increasing the concentration of external calcium or potassium in the bathing medium produces an increase in the contractility of the aorta up to a maximum which occurs in a normal Kreb's bicarbonate solution.

9. The evidence suggests a calcium-magnesium and a calcium-sodium competition at the arterial smooth muscle cell membrane.

10. The sodium ion concentration of the solution bathing the tissues can be decreased to at least 25% of normal without abolishing aortic contractility. If the sodium ion concentration is reduced to 50% of normal, there is a potentiation of contractility; if it is decreased to 25% of normal, there is a depression of contractility. This suggests

that, while lowering the sodium ion concentration slightly potentiates the arterial smooth muscle contractility, some external sodium is required for maximum contractility.

11. Increasing the external sodium ion concentration of the bathing medium depresses contractility, probably due to the simultaneous increase in the osmotic pressure of the solution. Increasing the osmotic pressure to a similar degree by the addition of sucrose had a similar depressant effect on the contractility.

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APPENDIX I

Preparation of Solutions

All solutions used to bathe the tissues were prepared in distilled water, which had been passed through a deionizer.

Normal Concentrated Salt Solutions:

Sodium Chloride (NaCl)	82.6 grams
Potassium Chloride (KCl)	4.22 "
Calcium Chloride ($CaCl_2$)	3.36 "
Potassium Phosphate (KH_2PO_4)	1.94 "
Magnesium Sulfate ($MgSo_4.7H_2O$)	3.50 "

This mixture of salts is dissolved in water to produce a total volume of one litre.

Kreb's bicarbonate solution is prepared by mixing 100 ml. of the concentrated salt solution, 900 ml. of distilled water, 192 ml. of 1.3% $NaHCO_3$ and 2.15 grams glucose.

Kreb's bicarbonate-free solution is prepared by mixing 100 ml. of the concentrated salt solution, 900 ml. of distilled water, 192 ml. of 0.92% NaCl and 2.15 grams glucose. The pH of this solution is adjusted to the desired level (pH 6.8 and 7.3) by the addition of 1 N NaOH.

Tris Buffered, Bicarbonate-Free Solutions

The pH of the bicarbonate-free solution is adjusted to the desired pH level (pH 6.8 and 7.3) by the addition of 5% Tris (Hydroxymethyl) aminomethane.

Variation of Calcium

The amount of $CaCl_2$ added to make the concentrated salt solution is adjusted to the required fraction of the normal amount. The amount of NaCl added is adjusted so that the amount of chloride in the system remains the same.

Variation of Potassium

The amount of potassium added to the concentrated salt solution is reduced to 1/2 or 1/4 of the normal amount in the 1/2 or 1/4 normal K solutions, respectively, by reducing the amount of KCl added. The amount of NaCl added is increased so that the total amount of chloride remains the same. When a 1/8 or 1/16 normal K solution is required, it is necessary to reduce the amount of KH_2PO_4 in the solution after all the KCl has been eliminated.

Variation of Magnesium

The $MgSO_4 \cdot 7H_2O$ is reduced or increased to the required fraction of the normal amount in the concentrated salt solution.

Variation of Sodium

Sucrose Kreb's is prepared by substituting 1.82 moles of sucrose (623 grams) for each mole (58.5 grams) of NaCl in the concentrated salt solution.

Choline chloride and lithium chloride Kreb's are prepared by substituting one mole of choline chloride (140 grams) or one mole of LiCl (42.4 grams) for each mole of NaCl (58.5 grams) in the concentrated salt solution.

The Kreb's solutions are prepared as described for the normal solutions above. The ionically adjusted concentrated salt solution is used in place of the normal concentrated salt solution.

APPENDIX II

Abbreviations

Dose - Noradrenaline Dose (gms./ml)

Response - Mean Response (measured in number of arbitrary units indicated on recording paper.)

S.E. - Standard Error

P - Probability (Student's Paired t-Test)

Tension - Number of grams of tension corresponding to "Response".

Note - Each set of data in each table was obtained from a separate experiment or group of experiments.

TABLE 1

WHOLE ANIMAL EXPERIMENTS
RESPIRATION WITH 5% CO_2 AND 30% CO_2

A.

NA Dose (ng/ml)	62.5		125		250		500		1 ugm/ml	
CO_2 Content of Gas	5%	30%	5%	30%	5%	30%	5%	30%	5%	30%
Mean Response (mm. Hg)	14.7	9.2	22	11.7	24.2	16.7	29.7	17.3	33.7	31
S.E.	0.35	1.31	1.73	1.99	0.79	1.58	4.75	2.49	3.54	4.94
P	0.05		0.05		0.05		0.015		0.30	

B.

NA Dose (ng/ml)	62.5		125		250		500		1 ugm/ml	
Mean Response (mm. Hg)			4.5	3	12.5	7.6	16.5	11.5	24.8	18.3
S.E.			1.66	1.08	2.72	1.84	3.00	2.74	4.26	4.33
P			0.25		0.18		0.16		0.28	

C.

NA Dose (ng/ml)	50		100		200		400		800	
Mean Response (mm. Hg)	13.3	11	19	16	31	21	39	31	56	42
S. E.	2.24	0.51	3.47	2.31	4.51	2.65	4.10	3.37	1.72	4.05
P	0.30		0.25		0.25		0.30		0.30	

A. Normal Cat. Perfused Abdominal Aorta (N=3)

B. Pithed Cat. Perfused Mesenteric Bed (N=4)

C. Ganglion Blocked Dog. Perfused Carotid Artery (N=3)

TABLE 2

TISSUES AERATED WITH 5% CO_2 AND 30% CO_2
PROCEDURE 1

	Dose gm/ml	5x10		$1x10^{-8}$		$5x10^{-8}$		$1x10^{-7}$		$5x10^{-7}$		$1x10^{-6}$		$5x10^{-6}$		$1x10^{-5}$	
A.	pH	7.41	6.78	7.5	6.78	7.46	6.87	7.39	6.87	7.47	6.70	7.39	6.8	7.43	7.0	7.47	6.85
	Response	0.8	0.4	1.1	1.1	4.9	6.9	8.1	9.8	11.7	11.5	15.7	16.2	19.5	18.7	20	19
	S.E.	0.23	0.09	0.13	0.16	0.60	0.30	0.60	1.04	0.82	0.81	0.89	0.65	0.85	1.05	1.04	1.18
	P	0.005		0.25		0.001				0.30		0.035		0.05		0.045	
	Tension	0.08	0.04	0.11	0.11	0.49	0.69	0.81	0.98	1.17	1.15	1.57	1.62	1.95	1.87	2.0	1.9
B.	pH	7.44	6.88	7.42	6.82	7.48	6.9	7.44	6.9	7.6	6.96	7.50	6.9	7.37	6.88	7.53	6.86
	Response	5.1	3.8	6.9	5.9	13	13.7	17	17.6	23.4	22.6	25.1	23.9	28.4	25.4	28.2	27.7
	S.E.	0.59	0.45	0.65	0.56	1.32	1.25	1.64	1.43	0.45	1.07	1.76	1.75	1.77	1.75	1.59	1.73
	P	0.005		0.004		0.005		0.07		0.045		0.004		0.001		0.001	
	Tension	0.51	0.38	0.69	0.59	1.3	1.37	1.7	1.76	2.34	2.26	2.51	2.39	2.84	2.54	2.82	2.77
C.	pH	7.5	6.82	7.33	6.73	7.5	6.83	7.5	6.8	7.47	6.8	7.47	6.82	7.43	6.83	7.5	6.8
	Response	2.3	1.4	4.3	2.9	9	10.6	11.7	12.8	18.2	17.3	20.8	19.8	22.7	23.7	24.2	26.1
	S.E.	0.61	0.31	0.83	0.69	1.47	1.39	0.69	1.48	2.32	2.10	2.51	2.49	2.84	2.64	2.60	2.26
	P	0.075		0.005		0.02		0.001		0.025		0.05		0.06		0.005	
	Tension	0.23	0.14	0.43	0.29	0.9	1.06	1.17	1.28	1.82	1.73	2.08	1.98	2.27	2.37	2.42	2.61

A. Normal Solution (N=8)

B. ½Ca Solution (N=8)

C. 1/4 Ca Solution (N=8)

TABLE 3

TISSUES AERATED WITH 5% CO_2 AND 30% CO_2
PROCEDURE 1

	Dose gm/ml	$5x10^{-9}$		$1x10^{-8}$		$5x10^{-8}$		$1x10^{-7}$		$5x10^{-7}$		$1x10^{-6}$		$5x10^{-6}$		$1x10^{-5}$	
A.	pH	7.43	6.89	7.55	6.95	7.61	6.86	7.58	6.95	7.55	6.90	7.50	6.98		6.91	7.31	6.87
	Response	1.25	1.69	3.18	3.13	7.6	8.6	10.4	9.9	10.4	11.7	13.5	14.3		15.9	17.8	19.2
	S.E.	0.28	0.38	0.38	0.62	1.16	1.23	1.30	1.18	1.23	1.35	1.92	2.02		1.86	1.76	2.61
	P	0.025		0.18		0.013		0.045		0.025		0.07				0.07	
	Tension	0.13	0.17	0.32	0.31	0.76	0.86	1.04	0.99	1.04	1.17	1.35	1.43		1.59	1.78	1.92
B.	pH					7.50	6.85	7.37	6.82	7.6	6.77	7.55	7.0	7.44	6.90	7.42	6.92
	Response					2	1.8	2.5	1.9	5.3	6.3	7.2	9.5	8.6	9.9	13.2	11.9
	S.E.					0.45	0.58	0.71	0.39	0.97	1.10	0.67	1.29	1.20	1.29	1.39	1.90
	P					0.45		0.45		0.04		0.001		0.025		0.06	
	Tension					0.2	0.18	0.25	0.19	0.53	0.63	0.72	0.95	0.86	0.99	1.32	1.19
C.	pH	7.42	6.95	7.5	6.89	7.48	7.00	7.45	7.04	7.44	6.89	7.34	6.88	7.32	6.97		6.90
	Response	2.5	1.1	3.4	5.3	6.2	8.8	14.3	13.1	18.2	19.2	18.7	16.7	21.5	17.5	19.1	21.1
	S.E.	0.22	0.09	1.09	1.58	1.02	0.57	0.31	0.22	0.78	0.63	0.86	0.90	1.03	1.19	0.83	0.77
	P	0.001		0.025		0.065		0.001		0.025		0.001		0.001		0.001	
	Tension	0.25	0.11	0.34	0.53	0.62	0.88	1.43	1.31	1.82	1.92	1.87	1.67	2.15	1.75	1.91	2.11

A. 1/8 Ca Solution (N=8)

B. 1/16 Ca Solution (N=5)

C. 1/2 K Solution (N=8)

TABLE 4

TISSUE AERATED WITH 5% CO_2 AND 30% CO_2
PROCEDURE 1

	Dose	$5x10^{-9}$		$1x10^{-8}$		$5x10^{-8}$		$1x10^{-7}$		$5x10^{-7}$		$1x10^{-6}$		$5x10^{-6}$		$1x10^{-5}$	
A.	pH	7.6	6.9		6.9	7.52	6.92	7.51	6.93	7.5	6.99	7.43	6.92	7.59	6.8	7.58	7.0
	Response	2.3	1.4	1.79	3.14	6.14	7.12	10.6	9	11.8	11.6	10.3	11.4	13.1	12.6	12.7	13.6
	S.E.	0.67	0.32	0.51	1.14	1.87	1.68	2.23	2.08	2.40	2.36	2.0	2.05	2.63	2.61	2.93	2.90
	P	0.09		0.06		0.035		0.02		0.08		0.09		0.035		0.21	
	Tension	0.23	0.14	0.18	0.31	0.61	0.71	1.06	0.9	1.18	1.16	1.03	1.14	1.31	1.26	1.27	1.36
B.	pH			7.51	6.99	7.38		7.62	6.77	7.46	6.94	7.45	6.95	7.43	6.91	7.43	6.91
	Response			0.8	0	3.7	5.9	5.1	4.1	10.4	11.9	13.6	14.6	16.2	15.8	17.7	16.6
	S.E.					0.78	0.87	0.61	1.06	1.23	1.65	1.63	1.68	1.99	2.23	1.92	1.89
	P					0.05		0.06		0.045		0.015		0.06		0.001	
	Tension			0.08	0	0.37	0.59	0.51	0.41	1.04	1.19	1.36	1.46	1.62	1.58	1.77	1.66
C.	pH					7.53	6.96	7.65	6.96	7.45	6.80	7.61	6.85	7.50	6.97	7.52	6.92
	Response					2.3	1.7	1.4	3.3	2.8	4.4	7	6.6	7.9	7.8	8.9	8
	S.E.					0.30	0.48	0.23	0.77	0.59	1.48	1.49	1.66	1.78	1.79	2.26	1.89
	P					0.03		0.05		0.075		0.04		0.15		0.35	
	Tension					0.23	0.17	0.14	0.33	0.23	0.44	0.7	0.66	0.79	0.78	0.89	0.80

A. 1/4 K Solution (N=7)

B. 1/8 K Solution (N=8)

C. 1/16 K Solution (N=8)

TABLE 5

TISSUES AERATED WITH 5% CO_2 AND 30% CO_2
PROCEDURE 1

	Dose	$5x10^{-9}$		$1x10^{-8}$		$5x10^{-8}$		$1x10^{-7}$		$5x10^{-7}$		$1x10^{-6}$		$5x10^{-6}$		$1x10^{-5}$	
A.	pH			7.58	6.96	7.57	6.97	7.67		7.58		7.64		7.63		7.63	6.91
	Response			3.3	4.6	7.1	9.2	9.1	8.5	13.8	15.4	15.6	15.3	16.7	1.6	14.1	14.5
	S.E.			0.42	0.79	0.51	0.77	0.64	0.60	0.72	0.99	1.22	1.28	1.15	1.24	1.35	1.36
	P			0.065		0.045		0.06		0.055		0.005		0.09		0.04	
	Tension			0.33	0.46	0.71	0.92	0.91	0.85	1.38	1.54	1.56	1.53	1.67	1.6	1.41	1.45
B.	pH					7.6	6.87	7.52	6.8	7.60	6.97	7.54	6.93	7.56	6.82	7.52	6.96
	Response					6.1	7.0	10.6	10.8	17.4	16.1	18.6	19.1	21.8	21.4	25.1	25.6
	S.E.					0.52	0.62	0.61	0.67	0.61	0.92	0.55	0.53	0.48	0.67	1.11	0.66
	P					0.055		0.50		0.075		0.045		0.06		0.065	
	Tension					0.61	0.70	1.06	1.08	1.74	1.61	1.86	1.91	2.18	2.14	2.51	2.56

A. Mg-free Solution (N=8)

B. 2x Mg Solution (N=8)

TABLE 6

TISSUES AERATED WITH 5% CO_2 AND 30% CO_2
PROCEDURE 1

	Dose	$5x10^{-9}$		$1x10^{-8}$		$5x10^{-8}$		$1x10^{-7}$		$5x10^{-7}$		$1x10^{-6}$		$5x10^{-6}$		$1x10^{-8}$	
A.	pH	7.56	6.90	7.48	6.97	7.53	6.90	7.53	6.97	7.60	6.89	7.57	6.90	7.47	6.98	7.46	6.96
	Response	1.06	2.44	3.4	3.2	7.7	10.1	12	13.9	18.3	18.4	21.1	19.8	23.9	23.1	24.4	23.8
	S.E.	0.22	0.39	0.17	0.20	0.55	0.57	0.76	0.72	1.04	0.94	0.94	0.81	1.11	0.98	1.36	0.90
	P	0.025		0.03		0.04		0.035		0.05		0.04		0.035		0.055	
	Tension	0.11	0.24	0.34	0.32	0.77	1.01	1.2	1.39	1.87	1.84	2.11	1.98	2.39	2.31	2.44	2.38
B.	pH					7.57	7.0	7.52	6.98	7.50	6.98	7.45	7.0	7.42	6.92	7.46	6.92
	Response					2.7	3.3	5.2	5.9	9.6	8.9	12	12.7	13.6	14.8	15.9	16.4
	S.E.					0.59	0.57	0.89	0.72	1.18	1.28	1.53	1.69	1.82	2.09	1.83	1.88
	P					0.015		0.02		0.015		0.025		0.01		0.045	
	Tension					0.27	0.33	0.52	0.59	0.96	0.89	1.2	1.27	1.36	1.48	1.59	1.64

A. 4x Mg Solution (N=8)

B. 2x K Solution (N=8)

TABLE 7

TISSUES AERATED WITH 5% CO_2 AND 30% CO_2
PROCEDURE II

Dose	$5x10^{-9}$		$1x10^{-8}$		$5x10^{-8}$		$1x10^{-7}$		$5x10^{-7}$		$1x10^{-6}$		$5x10^{-6}$		$1x10^{-5}$	
A. pH	7.36	6.75	7.32	6.72	7.34	6.77	7.31	6.87	7.40	6.83	7.41	6.90	7.42	6.87	7.48	6.75
Response	3.3	3.8	7.2	6.8	12.6	11.6	15.6	12	18.3	15	18.8	16.2	20	18.5	19.2	19
S.E.	0.94	1.04	1.09	1.04	0.6	1.81	2.13	1.92	2.08	1.99	2.23	2.14	2.11	2.13	2.26	2.12
P	0.025		0.005		0.005		0.005		0.005		0.005		0.015		0.005	
Tension	0.33	0.38	0.72	0.68	1.26	1.16	1.56	1.3	1.83	1.5	1.88	1.62	2.0	1.85	1.92	1.9
B. pH					7.49	6.89	7.38	6.90	7.46	6.88	7.42	6.90	7.32	6.87	7.48	6.92
Response					7.5	5.9	8.8	6.5	12.6	11.3	14.4	12.4	16.1	14.6	15.7	15.8
S.E.					1.22	0.90	1.42	0.86	1.29	1.08	1.13	1.21	1.22	1.18	1.09	1.26
P					0.001		0.001		0.001		0.001		0.002		0.001	
Tension					0.75	0.59	0.88	-.65	1.26	1.13	1.44	1.24	1.61	1.46	1.57	1.58
C. pH			7.25	6.77	7.20	6.89	7.30	6.85	7.42	6.98	7.53	6.95	7.51	6.91	7.43	6.84
Response			1.15	0.3	3.8	1.9	5.5	3.8	8.2	7.1	8.2	7.2	9.2	8.4	9.1	8.2
S.E.					0.41	0.97	1.21	0.95	1.52	1.61	1.59	1.55	1.97	1.96	1.98	1.89
P					0.001		0.001		0.001		0.001		0.005		0.001	
Tension			0.11	0.03	0.38	0.19	0.55	0.38	0.82	0.71	0.82	0.72	0.92	0.84	0.91	0.82

A. Normal Solution (N=16)

B. 1/2 Ca Solution (N=15)

C. 1/4 Ca Solution (N=10)

TABLE 8

TISSUES AERATED WITH 5% CO_2 AND 30% CO_2
PROCEDURE II

	Dose	$5x10^{-9}$		$1x10^{-8}$		$5x10^{-8}$		$1x10^{-7}$		$5x10^{-7}$		$1x10^{-6}$		$5x10^{-6}$		$1x10^{-5}$	
A.	pH			7.42	6.80	7.42	6.77	7.54	6.97	7.35	6.93	7.53	6.98	7.40	6.91	7.41	6.90
	Response			3.7	2.8	8.2	6.1	9.4	8.2	12.7	10.1	13.7	10.6	15.7	10.2	15.4	9.8
	S.E.			0.33	0.28	0.26	0.48	0.82	0.97	1.10	0.91	1.01	0.92	1.16	0.67	1.03	0.87
	P			0.035		0.001		0.001		0.001		0.001		0.001		0.001	
	Tension			0.37	0.28	0.82	0.61	0.94	0.82	1.27	1.01	1.37	1.06	1.57	1.02	1.54	0.98
B.	pH			7.57	6.94	7.48	6.83	7.33	6.96	7.54	6.94	7.40	6.96	7.40	6.93	7.50	6.92
	Response			2.3	0.5	8	5.6	9.4	7.2	11.4	8.9	12.8	8.1	14.3	9.4	14	9
	S.E.			0.24	0.37	0.76	0.16	1.01	0.66	1.0	0.83	1.14	0.80	1.16	0.53	1.18	0.62
	P			0.001		0.001		0.005		0.001		0.001		0.001		0.001	
	Tension			0.23	0.05	0.8	0.56	0.94	0.72	1.14	0.89	1.28	0.81	1.43	0.94	1.4	0.9
C.	pH	7.29	6.80	7.22	6.98	7.23	6.83	7.23	6.90	7.41	6.85	7.23	6.95	7.27	6.89	7.28	6.87
	Response	6.7	4.1	5.9	3.9	15.3	16.4	18.9	17.1	21.3	20.6	22.7	21.4	23.4	21.4	21.9	22.3
	S.E.	0.46	0.37	0.40	0.68	0.94	0.89	1.11	1.04	1.40	1.27	1.37	1.34	1.38	1.41	1.38	1.56
	P	0.005		0.01		0.02		0.001		0.17		0.001		0.045		0.09	
	Tension	0.67	0.41	0.59	0.39	1.53	1.64	1.89	1.71	2.13	2.06	2.27	2.14	2.34	2.14	2.19	2.23

A. 1/8 Ca Solution (N=15)

B. 1/16 Ca Solution (N=16)

C. 1/2 K Solution (N=7)

TABLE 9

TISSUES AERATED WITH 5% CO_2 AND 30% CO_2
PROCEDURE II

	Dose	$5x10^{-9}$		$1x10^{-8}$		$5x10^{-8}$		$1x10^{-7}$		$5x10^{-7}$		$1x10^{-6}$		$5x10^{-6}$		$1x10^{-5}$	
A.	pH	7.28	6.74	7.22	6.77	7.30	6.75	7.30	6.73	7.27	6.75	7.29	6.74	7.32	6.80	7.31	6.68
	Response	1.4	0.3	3.3	2.6	11.3	9.7	14.7	12.8	18.5	17.1	18.6	16.3	20.6	20.4	22.9	22
	S.E.	0.18	0.13	0.33	0.15	0.27	0.61	1.03	0.68	0.83	1.01	1.21	0.99	1.56	1.19	1.62	1.51
	P	0.004		0.04		0.008		0.005		0.008		0.001		0.001		0.01	
	Tension	0.14	0.03	0.33	0.26	1.13	0.97	1.47	1.28	1.85	1.71	1.86	1.63	2.06	2.04	2.29	2.2
B.	pH	7.40	6.90	7.58	6.97	7.44	6.85	7.44	6.85	7.45	6.84	7.51	6.88	7.30	6.86	7.20	6.73
	Response	5.2	5.4	8.5	7.7	12.5	11.6	11.4	12.3	17.8	15.6	17	15.9	17.7	17.2	18.2	17.3
	S.E.	0.73	0.77	0.79	0.96	1.09	1.46	1.76	1.87	1.66	1.78	1.49	1.56	1.47	1.46	1.18	1.45
	P	0.04		0.10		0.08		0.025		0.05		0.065		0.065		0.20	
	Tension	0.52	0.54	0.85	0.77	1.25	1.16	1.14	1.23	1.78	1.56	1.7	1.59	1.77	1.72	1.82	1.73
C.	pH					7.39	6.94	7.54	6.95	7.58	6.98	7.44	6.95	7.50	6.90	7.49	6.88
	Response			1.1	1.3	1.9	2.7	2.9	3.3	3.5	4.1	4.1	4.8	5.9	5.9	5.8	6.0
	S.E.					0.39	0.49	0.50	0.55	0.63	0.71	0.59	0.74	0.73	0.85	0.70	0.76
	P					0.001		0.001		0.005		0.001		0.002		0.002	
	Tension			0.11	0.13	0.19	0.27	0.29	0.33	0.35	0.41	0.41	0.48	0.59	0.59	0.58	0.60

A. 1/4 K Solution (N=8)

B. 1/8 K Solution (N=7)

C. 1/16 K Solution (N=14)

TABLE 10

TISSUES IN HIGH AND LOW pH, BICARBONATE FREE SOLUTIONS
PROCEDURE II

	Dose	$5x10^{-9}$		$1x10^{-8}$		$5x10^{-8}$		$1x10^{-7}$		$5x10^{-7}$		$1x10^{-6}$		$5x10^{-6}$		$1x10^{-5}$	
A.	pH	7.40	6.98	7.21	6.97	7.50	6.93	7.33	6.76	7.33	6.75	7.41	6.77	7.36	6.63	7.53	6.90
	Response	1.9	1.0	1.9	2.5	4.1	4.4	5.9	7.0	7.6	7.5	7.7	7.1	7.5	7.1	7.8	6.7
	S.E.	0.24	0.19	0.15	0.12	0.35	0.32	0.49	0.34	0.35	0.21	0.32	0.38	0.46	0.26	0.38	0.29
	P	0.015		0.002		0.01		0.02		0.5		0.015		0.01		0.01	
	Tension	0.19	0.10	0.19	0.25	0.41	0.44	0.59	0.70	0.76	0.75	0.77	0.71	0.75	0.71	0.78	0.67
B.	pH			7.32	6.71	7.31	6.73	7.36	6.70	7.21	6.75	7.28	6.83	7.34	6.71	7.23	6.71
	Response			0.7	0.8	5.1	4.2	6.9	6.6	11.1	10.6	12.6	11.8	14	13	15.2	13.8
	S.E.			0.17	0.24	0.59	0.69	0.49	1.14	0.66	0.58	0.71	0.83	0.79	0.93	0.96	1.07
	P			0.11		0.005		0.02		0.035		0.015		0.01		0.01	
	Tension			0.07	0.08	0.51	0.42	0.69	0.66	1.11	1.06	1.26	1.18	1.4	1.3	1.52	1.38
C.	pH			7.37	6.70	7.35	6.72	7.39	6.68	7.28	6.77	7.33	6.82	7.26	6.88	7.37	6.77
	Response			1.2	2.1	0	1.9	2.8	2.0	8.7	8.4	8.5	7.8	9.5	8.5	10.7	9.8
	S.E.							0.76	0.56	0.69	1.09	7.4	7.6	0.86	0.58	0.97	0.81
	P							0.045		0.12		0.045		0.015		0.075	
	Tension			0.12	0.21	0	0.19	0.28	0.20	0.87	0.84	0.85	0.78	0.95	0.85	1.07	0.98

A. Normal Solution (N=14)

B. 1/2 Ca Solution (N=15)

C. 1/4 Ca Solution (N= 8)

TABLE 11

TISSUES IN HIGH AND LOW pH, BICARBONATE FREE SOLUTIONS

Dose	$5x10^{-9}$		$1x10^{-8}$		$5x10^{-8}$		$1x10^{-7}$		$5x10^{-7}$		$1x10^{-6}$		$5x10^{-6}$		$1x10^{-5}$	
A. pH			7.44	6.91	7.59	6.95	7.47	6.95	7.44	6.92	7.58	6.90		6.6	7.33	6.75
Response			1.8	1.3	4.5	4.1	6.6	5.4	10.9	8.8	11.9	9.3	13.4	8.7	11.7	11.3
S.E.			0.44	0.41	0.34	0.88	1.18	0.95	1.69	1.08	0.48	0.40	0.60	1.03	1.18	1.36
P			0.005		0.003		0.001		0.015		0.001		0.003		0.045	
Tension			0.18	0.13	0.45	0.41	0.66	0.54	1.09	0.88	1.19	0.93	1.34	0.87	1.17	1.13
B. pH					7.29	6.79	7.35	6.80	7.48	6.82	7.3	6.81	7.36	6.89	7.6	6.87
Response					1.5	1.7	2.6	1.9	3.0	2.7	3.6	3.3	4	3.6	4	3.4
S.E.					0.21	0.28	0.37	0.33	0.42	0.45	0.45	0.45	0.47	0.35	0.42	0.46
P					0.15		0.007		0.04		0.09		0.50		0.001	
Tension					0.15	0.17	0.26	0.19	0.30	0.27	0.36	0.33	0.4	0.36	0.4	0.34
C. pH	7.39	6.99	7.35	6.95	7.48	6.85	7.23	6.93	7.34	6.86	7.41	6.92	7.40	6.98	7.40	6.96
Response	2.1	3.1	4.2	5.2	7.0	8.2	8.1	7.9	10.6	9.9	10.5	8.9	12.7	11.8	10.9	10.4
S.E.	0.46	0.59	0.50	0.92	0.73	0.82	0.71	1.18	1.04	1.04	2.33	2.28	2.72	1.32	1.30	1.21
P	0.50		0.025		0.003		0.15		0.50		0.15		0.01		0.30	
Tension	0.21	0.31	0.42	0.52	0.70	0.82	0.81	0.79	1.06	0.99	1.05	0.89	1.27	1.18	1.09	1.04

A. 1/8 Ca Solution (N=8)

B. 1/16 Ca Solution (N=8)

C. 1/2 K Solution (N=8)

TABLE 12

TISSUES IN HIGH AND LOW pH, BICARBONATE FREE SOLUTIONS

	Dose	$5x10^{-9}$		$1x10^{-8}$		$5x10^{-8}$		$1x10^{-7}$		$5x10^{-7}$		$1x10^{-6}$		$5x10^{-6}$		$1x10^{-6}$	
A.	pH	7.38		7.12	6.95	7.40	6.96	7.50	6.90	7.40	6.90	7.36	6.92	7.47	6.93		
	Response	2.13	2	2.13	2.8	3.1	4.1	5.5	5.2	4.9	4.6	6.3	7	5.3	7.2		
	S.E.	0.35	0.30	0.30	0.10	0.48	1.37	0.48	0.57	0.64	0.57	0.57	0.36	0.25	0.69		
	P	0.045		0.01		0.04		0.06		0.001		0.03		0.025			
	Tension	0.21	0.2	0.21	0.28	0.31	0.41	0.55	0.52	0.49	0.46	0.63	0.7	0.53	0.72		
B.	pH	7.50	6.90	7.61	6.93	7.59	6.85	7.59	6.85	7.61	6.84	7.63	6.82	7.30	6.86	7.20	6.73
	Response	5.2	5.4	8.5	7.7	12.5	11.6	11.4	12.3	17.8	15.6	17	15.9	17.7	17.2	18.2	17.3
	S.E.	0.73	0.80	0.79	0.96	1.20	1.47	1.88	1.77	1.66	1.27	1.51	1.44	1.48	1.47	1.18	1.46
	P	0.001		0.025		0.001		0.001		0.001		0.005		0.004		0.08	
	Tension	0.52	0.54	0.85	0.77	1.25	1.16	1.14	1.23	1.78	1.56	1.7	1.59	1.77	1.72	1.82	1.73
C.	pH					7.56	6.72	7.37	6.9	7.58	6.80	7.5	6.95	7.5	6.98	7.5	6.86
	Response					1.9	1.4	2	2.2	4.3	4.3	4.9	4.8	5.7	5.3	5.1	5.0
	S.E.					0.57	0.53	0.65	0.67	0.94	1.03	1.03	1.13	1.17	0.92	1.01	0.92
	P					0.15		0.10		0.20		0.09		0.09		0.13	
	Tension					0.19	0.14	0.2	0.22	0.43	0.43	0.49	0.48	0.57	0.53	0.51	0.50

A. 1/4 K Solution (N=8)

B. 1/8 K Solution (N=8)

C. 1/16 K Solution (N=6)

TABLE 13

TISSUES IN HIGH AND LOW pH, BICARBONATE FREE SOLUTIONS

Dose	$5x10^{-9}$		$1x10^{-8}$		$5x10^{-8}$		$1x10^{-7}$		$5x10^{-7}$		$1x10^{-6}$		$5x10^{-6}$		$1x10^{-5}$	
A. pH					7.23	6.76	7.33	6.78		6.85	7.28	6.7	7.38	6.63	7.28	6.62
Response					4.6	5.3	8.6	9.3	23.7	23.6	25.9	22.1	25	22.1	22.3	15.8
S.E.					0.80	1.25	3.31	3.23	1.41	0.66	1.56	1.33	1.77	1.24	1.73	0.81
P					0.055		0.035		0.001		0.001		0.005		0.001	
Tension					0.46	0.53	0.86	0.93	2.37	2.36	2.59	2.21	2.5	2.21	2.23	1.58
B. pH			7.38	6.89	7.33	6.8	7.34	6.90	7.35	6.87	7.33	6.98	7.32	6.83	7.40	6.98
Response			1.7	1.8	7.1	7.2	9.3	9.5	20.4	18.5	24.4	22.4	25.4	24.6	26.7	26.2
S.E.			0.07	0.24	0.65	0.52	1.69	1.12	0.58	0.46	0.39	0.73	0.32	0.42	0.40	0.24
P			0.005		0.005		0.04		0.005		0.05		0.005		0.005	
Tension			0.17	0.18	0.71	0.72	0.93	0.95	2.04	1.85	2.44	2.24	2.54	2.46	2.67	2.62
C. pH			7.4	6.9	7.33	6.83	7.46	6.98	7.48	6.98	7.45	6.81	7.47	6.77	7.30	6.98
Response			3.4	2.8	13.5	12.3	16.4	14.8	20.6	19.3	23.9	22.5	25.8	25	26.3	24.9
S.E.			0.84	0.73	0.73	1.10	0.71	0.88	0.85	1.15	0.82	0.76	0.63	0.76	0.85	0.91
P			0.02		0.03		0.005		0.001		0.045		0.004		0.001	
Tension			0.34	0.28	1.35	1.23	1.64	1.48	2.06	1.93	2.39	2.25	2.58	2.5	2.63	2.49

A. Mg-free Solution (N=8)

B. 2x Mg Solution (N=8)

C. 4x Mg Solution (N=8)

TABLE 14

TISSUES IN HIGH AND LOW pH, BICARBONATE FREE SOLUTIONS

	Dose	$5x10^{-9}$		$1x10^{-8}$		$5x10^{-8}$		$1x10^{-7}$		$5x10^{-7}$		$1x10^{-6}$		$5x10^{-6}$		$1x10^{-5}$	
A.	pH	7.23	7.0	7.30	6.83	7.2	6.94	7.55	6.85	7.44	6.85	7.44	6.9	7.3	6.82	7.3	6.87
	Response	1.3	2.8	2.4	3.6	10.3	8.4	8.5	15.2	17.8	17.2	20.8	19.4	21.8	20.6	21.9	21.2
	S.E.	0.16	0.12	0.47	0.32	0.35	0.77	0.69	0.94	0.91	1.21	0.84	1.12	1.02	1.00	1.25	1.06
	P	0.001		0.005		0.001		0.03		0.05		0.01		0.004		0.005	
	Tension	0.13	0.28	0.24	0.36	1.03	0.84	0.85	1.52	1.78	1.72	2.08	1.94	2.18	2.06	2.19	2.12
B.	pH					7.64	6.74	7.51	6.78	7.6	6.79	7.47	6.86	7.40	6.72	7.46	6.78
	Response					2.3	1.1	4.1	7.1	9.2	8.5	10.4	9.7	13	13	14.3	12.5
	S.E.					0.45	0.86	0.81	0.73	1.48	1.38	1.64	1.24	1.62	1.56	1.47	1.28
	P					0.01		0.006		0.007		0.25		0.008		0.15	
	Tension					0.23	0.11	0.41	0.73	1.48	1.35	1.64	1.24	1.62	1.56	1.47	1.28
C.	pH			7.59	6.74	7.61	6.72	7.56	6.87	7.46	6.84	7.65	6.85	7.46	7.00	7.58	7.02
	Response			2.6	0.25	2.4	1.9	4.3	3.1	5.8	3.0	6.9	6.6	8.1	7.9	8.4	8.1
	S.E.			0.57	0.09	0.25	0.16	0.53	0.28	0.55	0.38	0.62	0.48	0.62	0.78	0.79	0.68
	P			0.008		0.01		0.007		0.007		0.013		0.002		0.009	
	Tension			0.3	0.03	0.24	0.19	0.43	0.31	0.58	0.30	0.69	0.66	0.81	0.79	0.84	0.81

A. 2x K (N=8)

B. Normal, Tris-buffered Solution (N=8)

C. ½ Ca, Tris-buffered Solution (N=8)

TABLE 15

TISSUES AERATED WITH 5% CO_2 AND 30% CO_2
PROCEDURE I

Dose	$5x10^{-9}$		$1x10^{-8}$		$5x10^{-8}$		$1x10^{-7}$		$5x10^{-7}$		$1x10^{-6}$		$5x10^{-6}$		$1x10^{-5}$	
A. pH																
Response			1.6	2.4	7.3	7.0	12.1	10.8	15.3	18.8	17.8	21.2	25.3	23.1	26.5	25.1
S.E.			0.26	0.67	1.47	1.38	1.99	0.97	1.44	1.65	2.18	2.11	2.28	1.96	1.75	1.96
P			0.03		0.50		0.004		0.01		0.001		0.08		0.025	
Tension			0.16	0.24	0.73	0.70	1.21	1.08	1.53	1.88	1.78	2.12	2.53	2.31	2.65	2.51
B. pH					7.51	7.0	7.61	6.98	7.43	6.72	7.54	7.03	7.40	7.07		
Response					1.1	0.9	1.0	2.5	3.1	2.7	2.5	3.5	3.9	4.2		
S.E.					0.47	0.19	0.66	0.34	0.23	0.49	0.64	0.65	0.63	0.71		
P					0.001		0.001		0.15		0.008		0.25			
Tension					0.11	0.09	0.10	0.25	0.31	0.27	0.25	0.35	0.39	0.42		
C. pH	7.25	6.78	7.25	6.6	7.2	6.8	7.34	7.04	7.55	6.95	7.53	6.68	7.3	6.68	7.3	6.6
Response	1.3	1.9	1.5	2.5	5.6	5.3	5.1	5.6	8.6	7.0	10.7	10.5	14	13.5	10.4	14.8
S.E.	0.45	0.21	0.21	0.30	0.49	0.42	0.36	0.57	0.70	0.38	1.35	1.13	1.49	1.58	1.45	1.63
P	0.015		0.001		0.035		0.055		0.001		0.008		0.05		0.001	
Tension	0.13	0.19	0.15	0.25	0.56	0.53	0.51	0.56	0.86	0.70	1.07	1.05	1.4	1.35	1.04	1.48

A. 1/2 NaCl - 1/2 Sucrose Solution (N=8)

B. 1/2 NaCl - 1/2 LiCl Solution (N=8)

C. 1/4 NaCl - 3/4 Sucrose Solution (N=8)

TABLE 16

TISSUES IN HIGH AND LOW pH, BICARBONATE FREE SOLUTIONS

Dose	$5x10^{-9}$		$1x10^{-8}$		$5x10^{-8}$		$1x10^{-7}$		$5x10^{-7}$		$1x10^{-6}$		$5x10^{-6}$		$1x10^{-5}$	
A. pH					7.58	7.0	7.43	7.0	7.33	6.96	7.30	6.88	7.32	6.86	7.58	6.93
Response					2.9	1.9	6.8	4.7	11	10.6	15.9	12.1	18.4	15.6	18.9	19.3
S.E.					0.50	0.21	0.56	0.66	1.09	1.00	0.98	1.60	0.97	1.05	1.27	1.32
P					0.004		0.02		0.055				0.002		0.025	
Tension					0.29	0.19	0.68	0.47	1.1	1.06	1.59	1.21	1.84	1.56	1.89	1.93
B. pH					7.45	6.80	7.20	6.90	7.20	6.61	7.78	6.84	7.57	6.80	7.19	6.62
Response					1.1	0.9	1.9	1.5	2.3	2.3	2.9	2.2	2.5	3.8	5.4	2.9
S.E.					0.15	0.27	0.29	0.27	0.47	0.39	0.43	0.48	0.68	0.58	0.87	0.61
P					0.007		0.06		0.001		0.015		0.001		0.001	
Tension					0.11	0.09	0.19	0.15	0.23	0.23	0.29	0.22	0.25	0.38	0.54	0.29
C. pH			7.41	6.89	7.53	6.96	7.2	6.64	7.2	6.89	7.59	6.88	7.61	6.97	7.12	6.7
Response			2.4	3	8	8.4	10.6	9	13.7	14.7	19.6	13.1	21.8	23.2	21.5	15
S.E.			0.42	0.46	0.79	0.92	1.17	0.93	1.89	2.05	0.48	0.56	0.53	0.50	0.45	0.45
P			0.04		0.007		0.035		0.009		0.004		0.001		0.001	
Tension			0.24	0.3	0.8	0.84	1.06	0.9	1.37	1.47	1.96	1.31	2.18	2.32	2.15	1.5

A. 1/2 NaCl - 1/2 Sucrose Solution (N=8)

B. 1/2 NaCl - 1/2 LiCl Solution (N=8)

C. 1/4 NaCl - 3/4 Sucrose Solution (N=8)

TABLE 17

TISSUE CONTRACTILITY IN HYPERTONIC SOLUTIONS

	Dose	$5x10^{-9}$		$1x10^{-8}$		$5x10^{-8}$		$1x10^{-7}$		$5x10^{-7}$		$1x10^{-6}$		$5x10^{-6}$		$1x10^{-5}$	
A.	pH			7.52	6.81	7.26	6.87	7.28.	6.92	7.32	6.90	7.49	6.98	7.38	6.81		
	Response			2.1	1.9	2.5	2.0	2.8	2.4	2.9	4.9	4.3	4.3	4.3	5.3		
	S.E.			0.24	0.24	0.33	0.49	0.43	0.43	0.51	1.20	0.55	0.66	0.93	0.76		
	P			0.015		0.007		0.007		0.004		0.02		0.005			
	Tension			0.21	0.19	0.25	0.20	0.28	0.24	0.29	0.49	0.43	0.43	0.43	0.53		

	Solution	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
B.	Response					2.4	1.1	1.7	1.3	7.1	1.9	8.9	1.3	10.3	1.3	5.9	
	S.E.					0.18	0.20	0.19	0.16	0.87	0.28	0.97	0.21	0.90	0.25	0.83	
	P					0.045		0.045		0.04		0.035		0.025			
	Tension					0.24	0.11	0.17	0.13	0.71	0.19	0.89	0.13	10.3	0.13	0.59	

	Dose	$1x10^{-9}$			$1x10^{-8}$			$1x10^{-7}$			$1x10^{-6}$			$1x10^{-5}$		
C.	Solution	2	1	3	2	1	3	2	1	3	2	1	3	2	1	3
	Response							2.3	4.2	2.6	3.7	11.5	5.3	5.4	13	5.3
	S.E.							0.22	0.76	0.77	0.70	0.54	1.17	1.26	1.78	1.25
	P								0.10	0.07		0.10	0.08		0.10	0.19
	Tension							0.23	0.42	0.26	0.37	1.15	0.53	0.54	1.30	0.53

A. 2x Normal Na Solution. 5% CO_2 and 30% CO_2 (N=8)

B. Solution 1 - Normal Solution
2 - 1 Na - 1 Sucrose Solution (N=8)

C. Solution 1 - Normal Solution
2 - 1 Na - ½ Sucrose Solution
3 - 1½ Na Solution (N=6)

TABLE 18

TISSUE CONTRACTILITY IN SOLUTIONS WITH ALTERED SODIUM CONCENTRATIONS

		Dose	$1x10^{-9}$			$1x10^{-8}$			$1x10^{-7}$			$1x10^{-6}$			$1x10^{-5}$		
A.	Solution	2	1	3	2	1	3	2	1	3	2	1	3	2	1	3	
	Response				2.4	2.3	1.6	14.4	11.9	10.3	24.3	22.6	20.8	29.6	28.9	26.3	
	S.E.				0.56	0.27	0.26	1.08	1.01	0.97	1.34	1.30	1.80	1.98	1.75	1.25	
	P				0.12	0.05		0.02	0.04		0.06	0.05		0.12	0.15		
	Tension				0.24	0.23	0.16	1.44	1.19	1.03	2.43	2.26	2.08	2.96	2.89	2.63	

	Dose	$5x10^{-9}$		$1x10^{-8}$		$5x10^{-8}$		$1x10^{-7}$		$5x10^{-7}$		$1x10^{-6}$		$5x10^{-6}$		$1x10^{-5}$	
B.	Solution	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
	Response			0.6	1.0	1.9	2.7	3.0	3.4	5.4	6.6	6.4	8.5	10.6	9.9	10.1	10.2
	S.E.			0.38	0.51	0.19	0.20	0.33	0.45	0.47	0.48	0.51	0.46	0.50	0.59	0.41	0.42
	P			0.015		0.001		0.05		0.01		0.001		0.006		0.001	
	Tension			0.06	0.1	0.19	0.27	0.30	0.34	0.54	0.66	0.64	0.85	1.06	0.99	1.01	1.02
C.	Solution	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
	Response			1.1	1.4	2.4	3.3	5.7	5.9	8.9	6.9	10.1	7.9	12.0	8.4	9.0	8.2
	S.E.			0.16	0.12	0.17	0.22	0.25	0.40	0.45	0.51	0.61	0.34	0.63	0.41	0.35	0.37
	P			0.005		0.02		0.08		0.001		0.001		0.001		0.01	
	Tension			0.11	0.14	0.24	0.33	0.57	0.59	0.89	0.69	1.01	0.79	1.2	0.84	0.9	0.82

A. Solution 1 - Normal Solution
2 - ½ NaCl - ½ Choline Chloride Solution
3 - 1/4 NaCl - 3/4 Choline Chloride Solution (N=8)

B. Solution 1 - 1/8 Ca - 1 Na Solution
2 - 1/8 Ca - 1/2 Na Solution + Sucrose (N=8)

C. Solution 1 - 1/8 Ca - 1 Na Solution
2 - 1/8 Ca - 1/4 Na Solution + Sucrose (N=8)

TABLE 19

TISSUE CONTRACTILITY IN SOLUTIONS WITH ALTERED MAGNESIUM CONCENTRATIONS

Dose	$1x10^{-9}$		$5x10^{-9}$		$1x10^{-8}$		$5x10^{-8}$		$1x10^{-7}$		$5x10^{-7}$		$1x10^{-6}$		$5x10^{-6}$	
A. Solution	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Response	3.5	2.0	4.6	5.4	11.3	12.7	15.8	16.8	21.6	22.6	20.9	24.4	23.4	25.5	23.5	23.8
S.E.	0.59	0.32	0.72	0.72	0.70	0.96	1.00	1.12	0.77	0.43	0.96	0.73	0.70	0.76	2.14	0.88
P	0.02		0.005		0.01		0.009		0.009		0.08		0.001		0.07	
Tension	0.35	0.20	0.46	0.54	1.13	1.27	1.58	1.68	2.16	2.26	2.09	2.44	2.34	2.55	2.35	2.38
B. Solution	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Response			1.36	0.79	4.8	4.1	7.8	7.6	14.4	14.1	18.0	17.1	22.7	22.3	24.5	23.4
S.E.			0.30	0.17	0.44	0.53	1.03	0.81	1.33	0.74	1.73	1.73	2.27	2.19	2.43	2.29
P			0.09		0.09		0.09		0.011		0.05		0.14		0.04	
Tension			0.14	0.08	0.48	0.41	0.78	0.76	1.44	1.41	1.8	1.71	2.27	2.23	2.45	2.34

A. Solution 1 - Normal Solution
2 - Mg-free Solution (N=8)

B. Solution 1 - Normal Solution
2 - 4x Mg Solution (N=8)

TABLE 20

AORTA CONTRACTILITY WITH DIFFERENT CALCIUM CONCENTRATIONS IN THE BATHING MEDIUM

Ca^{+}	0	1/16 normal	1/8 normal	1/4 normal	1/2 normal	normal	2 x normal
	Noradrenaline Dose = 5×10^{-7} gm/ml. (500 ng/ml)						
Mean Response (gms. tension)	0.40	0.46	0.60	0.71	0.74	0.94	0.89

TABLE 21

AORTA CONTRACTILITY WITH DIFFERENT POTASSIUM CONCENTRATIONS IN THE BATHING MEDIUM

K^+	1/16 normal	1/8 normal	1/4 normal	1/2 normal	normal	2 x normal	2 x normal
	Noradrenaline Dose = 1 x 10^{-7} gm/ml. (100 ng/ml)						
Mean Response	0.61	0.75	0.80	0.96	1.11	0.94	1.05
	Noradrenaline Dose = 5 x 10^{-7} gm/ml. (500 ng/ml)						
Mean Response	1.26	1.01	1.28	1.21	1.29	1.55	1.54

TABLE 22

COMPARISON OF MAXIMUM TENSION LEVELS
ATTAINED BY AORTA LOOPS IN THE PRESENCE AND
ABSENCE OF BICARBONATE IN THE BATHING MEDIA

Solution	Maximum Levels of Tension (grams)	
	With HCO_3^-	Without HCO_3^-
Normal	2.00	0.78
½ Ca	2.23	1.52
1/4 Ca	1.77	1.07
1/8 Ca	1.73	1.34
1/16 Ca	1.36	0.40
½ K	2.50	1.27
1/4 K	1.80	0.72
1/8 K	1.80	1.82
1/16 K	0.75	0.57
2 x K	1.64	2.19
Mg-free	1.67	2.59
2 x Mg	2.56	2.67
4 x Mg	2.44	2.63
Mean Response	1.87	1.51